EXPERIENTIA

EST EXPERIENTIA SCIENTIA PARACELSUS

REVUE MENSUELLE DES SCIENCES PURES ET APPLIQUÉES
MONATSSCHRIFT FÜR DAS GESAMTE GEBIET DER NATURWISSENSCHAFT
RIVISTA MENSILE DI SCIENZE PURE E APPLICATE
MONTHLY JOURNAL OF PURE AND APPLIED SCIENCE

Editores:
P. HUBER · R. MATTHEY · A. v. MURALT · L. RUZICKA
Basel Lausanne Bern Zürich

Redactor: H. MISLIN, Basel-Mainz

BIRKHÄUSER VERLAG BASEL
SUISSE – SCHWEIZ – SVIZZERA – SWITZERLAND

SEP 27 '56
UNIVERSITY OF HAWAII
LIBRARY

SOMMAIRE - INHALT - SOMMARIO - CONTENTS

Brèves communications - Kurze Mitteilungen - Brevi comunicazioni - Brief Reports

A. Katz: Nachweis von Scilliglaucosidin in den Zwiebeln von *Bowiea volubilis* Harvey 285
L. Darcis, P. Hotterbeex et C. Onkelinx: Action du becaptan (β-mercapto-éthylamine) sur la radiosensibilité des cellules vaginales du rat femelle 286
F. H. Schwarzenbach: Untersuchungen über einen mikrobiologisch feststellbaren Serumfaktor nach Gelbsucht 289
M. O. Tirunarayanan and W. A. Vischer: Effect of Vitamins on the Acute Toxicity of Hydrazine Derivates . . 291
P. A. J. Janssen and A. Jageneau: Mydriatic Activity of Analgesics in Mice 293
E. F. Lüscher: Glukose als Cofactor bei der Retraktion des Blutgerinnsels 294
B. Exer: Über die Wirkung von Phenylbutazon auf den Tricarbonsäurezyklus und andere Fermentsysteme . . . 294
B. Holmqvist and O. Oscarsson: Synaptic Connections of Ib Muscle Afferents to Ventral Spinocerebellar Tract Neurons 296
G. Benz: Der Trockenheitssinn bei Larven von *Drosophila Melanogaster* 297
Jane Mayr: Advantages Given by P^{33}–or Aged P^{32}–in Autoradiography 298
U. Söderberg: Changes in Muscle Circulation Elicited by Local Heating of Hypothalamus 299
L. De Caro, G. Rindi, V. Perri, and G. Ferrari: Effects of Pyrithiamine and Oxythiamine on the Thiamine Content of Tissues and Blood Pyruvate in Mice 300
K. S. Ambe and Kamala Sohonie: Trypsin Inhibitor in Plant Metabolism 302
R. Caravaglios and P. Chiaverini: Paper Electrophoresis of the Soluble Proteins of the Central Nervous Tissue 303
N. Farinella-Ferruzza: The Transformation of a Tail into Limb After Xenoplastic Transplantation 304
V. Caglioti, A. Ascenzi, and A. Santoro: On the Interpretation of the Low-Angle Scatter of X-Rays from Bone Tissue 305
M. Durante: Cholinesterase in the Development of *Ciona intestinalis* (Ascidia) 307
A. Schatz, K. E. Karlson, and J. J. Martin: Trace Element Stimulation of Keratin (Hair) Degradation by Oral Keratinolytic Microflora 308
H. Barnes: The Biometry of the Cirripede, *Chthamalus stellatus* (Poli) 309
M. Polster: The Exchange of Phosphorus in Shells of the Aquatic Snail *Physa acuta* 310
L. Mosonyi, L. Pollák, R. Zulik, and Gy. Károlyházi: Streptomycin and Endocrine System 311
A. A. Hakim: Diagnostic Test for Cancer 313
J. Moutschen, Z. M. Bacq et A. Herve: Action du rayonnement X sur la croissance de la plantule d'orge 314
E. Schreider: Relations entre les taux cétoniques du plasma et des organes 315
S. Reuter and K. R. S. Ascher: The Action of Di-(*p*-chlorophenyl) trichloromethylcarbinol (DTMC) and Di-(*p*-chlorophenyl) trifluoromethylcarbinol on Houseflies 316
F. H. Sobels: The effect of Formaldehyde on the Mutagenic Action of X-rays in *Drosophila* 318
E. Wolff: La culture de cellules tumorales sur des explants d'organes *in vitro* 321
S. W. Mayer and M. E. Morton: Tumor Necrosis After the Intra-Tumor Injection of Chemical Agents and Colloidal Radioisotopes 322
J. A. Schneider and Marie McArthur: Potentiation Action of Ibogaine (Bogadin TM) on Morphine Analgesia 323

EXPERIENTIA

L'EXPERIENTIA publiera:

1° des *articles originaux* sur les récentes recherches scientifiques écrits dans une des langues principales;

2° de *brèves communications;*

3° informera ses lecteurs des événements marquants de la vie scientifique, donnera des *comptes rendus concernant les récentes publications, les congrès et les assemblées.*

Die EXPERIENTIA stellt sich die Aufgabe:

1. durch *zusammenfassende Originalartikel* in einer der wissenschaftlichen Hauptsprachen von Autoren aus verschiedenen Ländern über Forschungsergebnisse berichten zu lassen, die im Vordergrund des Interesses stehen;
2. kurze *Mitteilungen* aufzunehmen;
3. durch Besprechung neuerschienener *Bücher*, durch Referate über *Kongresse* und *Versammlungen* sowie durch andere Mitteilungen über die bedeutendsten Ereignisse des naturwissenschaftlichen Lebens zu informieren.

EXPERIENTIA si propone di pubblicare:

1. *articoli originali riassuntivi*, in una delle principali lingue usate dalla scienza, ad opera di autori di diversi paesi, su risultati scientifici di grande interesse;
2. *brevi comunicazioni;*
3. *recensioni di nuovi libri, relazioni di congressi e riunioni*, come pure altre comunicazioni su importanti avvenimenti nel campo delle scienze naturali.

The aim of EXPERIENTIA is:

1. to publish comprehensive articles embodying the results of recent scientific research. These will be written in one of the principal scientific languages and contributed by authors in various countries;
2. to publish *brief reports;*
3. to give information about the most important events in natural science by means of *reviews of the latest books, reports on congresses and meetings*, as well as through other communications.

E X P E R.

L'EXPERIENTIA paraît le 15 de chaque mois. Vente et abonnement dans toutes les librairies suisses et étrangères, ou directement chez l'éditeur. Abonnement pour un an Fr. 36.– pour la Suisse; pour l'étranger fr. 46.–. Ces prix s'entendent en francs suisses.
Adresser toute correspondance touchant la rédaction de l'EXPERIENTIA exclusivement à l'éditeur soussigné.
Dernier délai d'admission pour les manuscrits: 2 mois avant la parution. Les auteurs recevront gratuitement, s'ils le désirent, 50 tirés à part de format 14,5 sur 21 cm, sans couverture. Pour le prix d'un nombre plus grand et pour la couverture s'adresser à l'éditeur. Les tirages à part doivent être commandés *avant* l'impression du périodique.
Prix pour les annonces: $^1/_1$ page fr. 220.–, ½ page fr. 132.–, ¼ page fr. 77.–. Placements spéciaux: prix sur demande. Demandes à l'éditeur.
L'EXPERIENTIA est imprimée en Suisse.

Editions Birkhäuser S.A., Bâle 10 (Suisse), Elisabethenstrasse 15
Adresse télégraphique: Edita Bâle

EXPERIENTIA esce al 15 di ogni mese e può esser richiesta ad ogni libreria svizzera o estera, o anche direttamente alla casa editrice. L'abbonamento annuo è di fr. 36.– per la Svizzera; all'estero fr. 46.–. I prezzi vanno intesi valuta svizzera.
Tutti gli invii alla redazione di EXPERIENTIA vanno indirizzati esclusivamente alla sottoindicata casa editrice.
La redazione di ogni fascicolo si chiude 2 mesi prima della pubblicazione.
Gli autori ricevono, su desiderio, 50 estratti del formato 14,5 × 21 cm, senza copertina. Il prezzo degli estratti in più e della copertina viene indicato, su richiesta, dalla casa editrice. Gli estratti vanno ordinati *prima* della stampa della Rivista.
Prezzi per annunci: $^1/_1$ pag. fr. 220.–, ½ pag. fr. 132.–, ¼ pag. fr. 77.–; per pagine speciali, accordi da stabilire. Gli annunci sono da inviare alla casa editrice.
EXPERIENTIA si stampa in Isvizzera.

Casa editrice Birkhäuser S.A., Basilea 10 (Svizzera), Elisabethenstr. 15
Indirizzo telegrammi: Edita Basilea

Die EXPERIENTIA erscheint am 15. jedes Monats und kann im In- und Auslande durch jede Buchhandlung oder direkt vom Verlag bezogen werden. In Algerien, Belgien, Dänemark, Deutschland, Finnland, Frankreich, Italien, Luxemburg, Marokko, Norwegen, Österreich, Portugal, Schweden nehmen auch die Postämter Bestellungen entgegen. Der Abonnementspreis beträgt in der Schweiz Fr. 36.–, im Ausland sFr. 46.–.
Alle Zuschriften an die Redaktion sind ausschliesslich an den Verlag zu richten. Redaktionsschluss 2 Monate vor Erscheinungsdatum.
Die Autoren erhalten auf Wunsch 50 Gratisseparata im Format 14,5 × 21 cm, ohne Umschlag. Die Kosten für weitere Separata und für Umschläge sind beim Verlag zu erfragen. Separata sind *vor* dem Druck der Zeitschrift zu bestellen.
Insertionspreise: $^1/_1$ Seite Fr. 220.–, ½ Seite Fr. 132.–, ¼ Seite Fr. 77.–; für Vorzugsseiten besondere Vereinbarung. Inseratenannahme durch den Verlag.
Die EXPERIENTIA wird in der Schweiz gedruckt.

Birkhäuser Verlag AG., Basel 10 (Schweiz), Elisabethenstrasse 15
Telegrammadresse: Edita Basel

EXPERIENTIA is published on the 15th of every month and can be obtained in any country through the booksellers or from the publishers. The annual subscription price by inland mail is fr. 36.–; other countries fr. 46.–. Prices in Swiss currency. All communications to the editors should be addressed to the publishers. All manuscripts for publication in a given number must be in the hands of the publishers 2 months before the publication.
The authors receive, on request, 50 reprints 14,5 × 21 cm without cover free of charge. For the prices of additional reprints and covers, inquiries should be addressed to the publishers. Reprints must be ordered *before* the number is printed.
Prices for advertising: $^1/_1$ page fr. 220.–, ½ page fr. 132.–, ¼ page fr. 77.–. Advertisements should be sent to the publishers.

Published by Birkhäuser Ltd., Basle 10 (Switzerland), Elisabethenstr. 15
Telegrams: Edita Basle

Printed in Switzerland / Birkhäuser AG. Basel

Verzeichnis der Inserenten – Liste des annonceurs – List of Advertisers – Experientia XII/8

Birkhäuser Verlag, Basel
Ciba AG., Basel
Sandoz AG., Basel
Ganz & Co., Zürich
Lonza AG., Basel

Im Herbst 1956 erscheint

VON BUDDENBROCK

Vergleichende Physiologie

BAND III

ERNÄHRUNG, WASSERHAUSHALT UND MINERALHAUSHALT DER TIERE

Von Prof. Dr. *W. von Buddenbrock*, Universität Mainz. Ca. 700 Seiten mit 207 Abbildungen. Ganzleinen Fr./DM 66.–, broschiert Fr./DM 62.–
(Sammlung «Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften» – Reihe der experimentellen Biologie, Band 8)

Aus dem Inhalt: A. Ernährung und Verdauung: Einleitung und allgemeiner Teil – Aminosäuren – Vitamine – Die spezielle Ernährungsweise der Tiere – Die Nahrungsaufnahme – Die Fermente – Die Aufnahme der Nahrung durch den Darm – Die Ernährung mit Hilfe von Symbionten – Die einzelnen Tierstämme der Wirbellosen – Protozoen – Cnidarier – Echinodermen – Würmer – Crustaceen – Arachnoideen – Insekten – Mollusken – Tunikaten und Branchiostoma – Wirbeltiere – Säugetiere – Vögel – Reptilien – Amphibien – Fische. B. Wasserhaushalt und Mineralhaushalt: Der Wasserhaushalt – Landtiere – Wassertiere – Der Wasserhaushalt der einzelnen Tierstämme – Protozoen – Coelenteraten – Würmer – Onychophoren – Crustaceen – Arachnoideen – Insekten – Mollusken – Fische – Amphibien – Reptilien – Vögel – Säugetiere – Der Mineralhaushalt – Alkalimetalle – Calcium – Magnesium – Zink – Mangan – Eisen – Kupfer – Kobalt – Vanadium.

Das Buch ist kein Handbuch und Nachschlagewerk, sondern ein erweitertes Lehrbuch, das besonders dem jüngeren Fachzoologen, der sich in das Gebiet der Zoophysiologie einarbeiten will, ein unentbehrlicher Ratgeber sein wird. Es bringt eine überwältigende Stoff-Fülle und eine beinahe unabsehbare Literatur. Es gibt in keiner anderen Sprache etwas Ähnliches.

Bestellungen an Ihren Buchhändler

BIRKHÄUSER VERLAG · BASEL / STUTTGART

BECKMAN

AUTOMATISCHES
QUARZ-SPEKTROPHOTOMETER

MODELL

DK-2

- Lineare Wellenlänge-Darstellung Ultraviolett bis Nah-Infrarot **185–3500 mμ**
- Wahlweise lineare Aufzeichnung in Transmission und Extinktion
- Differentialmessungen an nahezu gleichen Proben
- Auflösung: 0,3 mμ im UV
  1 mμ im Sichtbaren
  3 mμ im IR
- Doppelstrahlprinzip kombiniert mit den Vorteilen des Einstrahlverfahrens
- Empfänger: Pbs-Zellen modernster Konstruktion. 1P28 Photomultiplier
- Wechselstromnetzanschluß 220 V 50 Hz. Eingebaute hochwirksame Stabilisation

Zuverlässige Beratung und Service durch eigenes Fachpersonal!

Bitte verlangen Sie Prospekt Nr. 377/E

GANZ & CO
BAHNHOFSTR. 40
TEL. (051) 23 97 73
Zürich

LONZA

Calcium-Carbid

Metall-Legierungen: Ferrosilicium. Ferrosilicium-Aluminium (Alsimin), Ferrosilicium-Mangan.

Silicium-Metall (Reinsilicium)

Silicium-Carbid für schleif- und feuerfeste Zwecke sowie «Lonsicar» als Betonhartstoff. **Graphit.**

Stickstoff- und Komplex-Dünger: Kalksalpeter, Ammonsalpeter, Ammonsulfat, Kalkstickstoff, Nitrophosphat, Nitrophosphatkali, Volldünger, Composto Lonza.

Kunststoffe: Polyvinylchlorid, Polyvinylacetat, Mischpolymerisate, Polyvinylalkohol

Weitere chemische Produkte

Acetaldehyd
Acetessiganilid
Acetessigäthylester
Acetessigmethylester
Aethylacetat
Ammoniak
Ammonnitrat
Anilin
Butylalkohol
Crotonaldehyd
Dicyandiamid
Essigsäure
Essigsäureanhydrid
Formaldehyd
Harnstoff
Methylacetat
Methylalkohol
Natriumacetat
Natriumnitrat
Natriumnitrit
Nitriersäure
Nitrobenzol
Paraldehyd
Salpetersäure
Speziallösungsmittel
Vinylacetat monomer

ELEKTRIZITÄTS-
WERKE
UND
CHEMISCHE
FABRIKEN
A.G.
BASEL

EXPERIENTIA

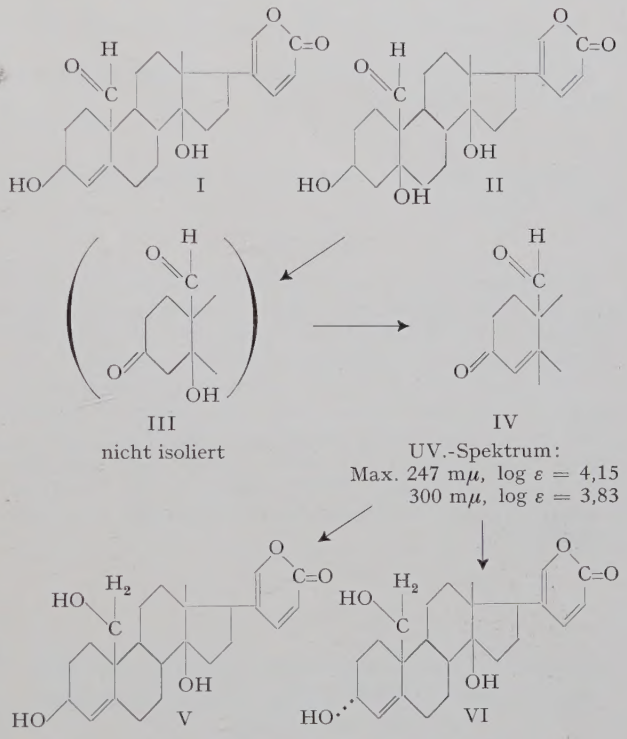
Vol. XII - Fasc. 8 Pag. 285–324 15. VIII. 1956

Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. – Für die kurzen Mitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed by their correspondents.

Nachweis von Scilliglaucosidin in den Zwiebeln von *Bowiea volubilis* HARVEY[1,2,3]

Zu Vergleichszwecken (siehe weiter unten) wurde Scilliglaucosidin-19-ol (V[4]) und 3-Epi-Scilliglaucosidin-19-ol (VI) benötigt. Scilliglaucosidin hat nach STOLL, VON WARTBURG und RENZ die Formel I[4], ist also ein 5-Anhydro-derivat von Hellebrigenin (II)[5]. Zur Darstellung von V und VI wurde deshalb Hellebrigenin[5] mit Pt und O_2 (vgl. Dehydrierung von Ouabagenin[6] und Dihydro-ouabagenin[7]) in Aceton-Wasser zum 3-Dehydro-hellebrigenin III dehydriert. Dieses wurde nicht isoliert, sondern durch Kochen mit Eisessig in 5-Anhydro-3-dehydro-hellebrigenin (IV) übergeführt. Reduktion von IV mit $NaBH_4$[8] gab nach chromatographischer Reinigung an Al_2O_3 zwei kristallisierte, papierchromatographisch einheitliche Substanzen. Sie zeigten mit 84%iger H_2SO_4 dieselbe Farbreaktion (rot → mauve), unterschieden sich jedoch deutlich im Papierchromatogramm (stationäre Phase: Wasser; mobile Phase: Toluol-Butanol 95:5; Sichtbarmachen der Flecken mit *p*-Dimethylaminobenzaldehyd-Schwefelsäure-Reagens[9]; beide Flekken orange; Verhältnis der Laufstrecken etwa 1:2). Die im Papierchromatogramm rascher laufende Substanz zeigt ein Nujol dasselbe IR.-Spektrum[10] wie Scilliglaucosidin-19-ol (V)[11]. Auch lief sie im Papierchromatogramm genau gleich wie letztgenanntes, welches zu diesem Vergleich durch $NaBH_4$-Reduktion[8] von 1 mg authentischem Scilliglaucosidin (I) hergestellt wurde[12]. Die beiden genannten Farbreaktionen von V und VI aus Hellebrigenin und von V aus authentischem Scilliglaucosidin waren ebenfalls genau gleich. Die Identität von V aus Hellebrigenin mit Scilliglaucosidin-19-ol (V) ist somit gesichert. Auf Grund der Farbreaktionen und der Reaktionsfolge ist anzunehmen, dass VI 3-Epi-Scilliglaucosidin-19-ol ist [13].



Die Aldehydgruppe im Scilliglaucosidin (I) wurde von STOLL *et al.*[4] aus Analogiegründen (unter anderem auf Farbreaktionen basierend[14]) an C-10 angenommen. Da die Stellung der Aldehydgruppe in Hellebrigenin (II) durch dessen Überführung in Strophanthidinabbauprodukte gesichert ist[5], leistet die Überführung von II in V den strengen Beweis für die C-10-Stellung der Aldehydgruppe in Scilliglaucosidin (I).

Kürzlich wurde die Isolierung eines Bufadienolidgenins $C_{24}H_{32}O_5$ aus Zwiebeln einer Variante von *Bowiea volubilis* HARVEY mitgeteilt. Die vorläufig Nabogenin genannte Substanz zeigte grosse Ähnlichkeit mit Scilli-

[1] 8. Mitteilung über die Glykoside von *Bowiea vocubilis* HARVEY und 162. Mitteilung über Glykoside und Aglykone.

[2] 7. Mitt.: A. KATZ, Helv. chim. Acta *38*, 1565 (1955).

[3] 161. Mitt.: H. KÜNDIG-HEGEDÜS und O. SCHINDLER, Helv. chim. Acta *39*, 904 (1956).

[4] A. STOLL, A. VON WARTBURG und J. RENZ, Helv. chim. Acta *36*, 1531 (1953); *36*, 1538 (1953).

[5] J. SCHMUTZ, Helv. chim. Acta *32*, 1442 (1949).

[6] C. MANNICH und G. SIEWERT, Ber. dtsch. chem. Ges. *75*, 737 (1942).

[7] R. P. A. SNEEDEN und R. B. TURNER, J. Amer. chem. Soc. *77*, 130 (1950).

[8] A. HUNGER und T. REICHSTEIN, Ber. dtsch. chem. Ges. *85*, 635 (1952).

[9] A. KATZ, Pharm. Acta Helv. *29*, 369 (1954).

[10] Aufgenommen von Herrn Dr. P. ZOLLER, Basel, auf einem Perkin-Elmer-Spektrophotometer, Modell 21.

[11] A. STOLL, A. VON WARTBURG und J. RENZ, Helv. chim. Acta *36*, 1538 (1953).

[12] A. STOLL, A. VON WARTBURG und J. RENZ führten Scilliglaucosidin durch Reduktion nach MEERWEIN-PONNDORF in Scilliglaucosidin-19-ol über. Herrn Prof. Dr. A. STOLL, Basel, sei auch hier für die freundliche Überlassung von Scilliglaucosidin herzlich gedankt.

[13] Reduktion von Δ^4-Cholestenon mit $LiAlH_4$ führt ebenfalls zu den zwei an C-3 epimeren Δ^4-Cholestenolen: H. MC. KENNIS und G. W. GAFFNEY, J. biol. Chem. *175*, 217 (1948). – PL. A. PLATTNER, H. HEUSSER und A. B. KULKANI, Helv. chim. Acta *32*, 265 (1949).

[14] A. STOLL, A. VON WARTBURG und J. RENZ, Helv. chim. Acta *36*, 1557, 1565 (1953).

glaucosidin (besonders waren die IR.-Spektren der Azetate praktisch identisch), unterschied sich von diesem jedoch in der Farbreaktion mit 84%iger H_2SO_4 und in der optischen Drehung etwas. Es war zu vermuten, dass entweder das 3-Epimere des Scilliglaucosidins oder unreines Scilliglaucosidin vorlag. Gegen die letztgenannte Auffassung sprach die Tatsache, dass Nabogenin chromatographisch gereinigt war und im Papierchromatogramm nur einen Flecken zeigte. Zur Abklärung wurde nun Nabogenin mit $NaBH_4$ reduziert. Das rohe Reduktionsprodukt gab nach chromatographischer Reinigung an Al_2O_3 Kristalle, die sich auf Grund der Farbreaktionen, des Verhaltens im Papierchromatogramm und des IR.-Spektrums[10] in Nujol als identisch mit V aus I und II erwiesen. Da sich gemäss obigen Ausführungen auch die Formel von 3-Episcilliglaucosidin für Nabogenin ausschliessen lässt, folgt, dass letzteres ein durch Chromatographie an Al_2O_3 nicht trennbares Gemisch war, das vorwiegend aus Scilliglaucosidin (I) bestand; Auftrennung des Gemisches gelang jedoch nach Reduktion der Aldehyd- zur Oxymethylgruppe.

Ich danke Herrn Prof. Dr. T. Reichstein für das meiner Arbeit entgegengebrachte Interesse.

Herrn W. Zürcher sei für die gewissenhafte Hilfe bei der Ausführung der Versuche gedankt.

A. Katz

Organisch-chemische Anstalt der Universität Basel, den 3. Juni 1956.

Summary

Hellebrigenin was converted to Scilliglaucosidin-19-ol and 3-Epi-Scilliglaucosidin-19-ol. This proves the correctness of the assumption of Stoll *et al.*[3] that the aldehydic group of Scilliglaucosidin is in position C-10.

Comparison of a reduction product of Nabogenin, a substance isolated earlier from the bulbs of *Bowiea volubilis* Harvey, with Scilliglaucosidin-19-ol and 3-Epi-Scilliglaucosidin-19-ol showed its identity with the first named compound. This proves that the so called Nabogenin was a mixture consisting mainly of Scilliglaucosidin.

Action du becaptan (β-mercapto-éthylamine) sur la radiosensibilité des cellules vaginales du rat femelle

I. – *Introduction.* 1° Depuis 1946, nombre de chercheurs et en particulier ceux de l'Ecole de Liège se sont attachés à découvrir des substances chimiques susceptibles de protéger des organismes vivants contre les radiations ou les poisons radiomimétiques.

L'ensemble des travaux sur ces substances et leurs propriétés ont été excellemment étudiés et synthétisés par Bacq et Alexander[1]. Il semble bien que ce soit la cystéamine ou β-mercapto-éthylamine commercialisée par la firme Labaz sous le nom de Becaptan qui ait jusqu'à présent donné le plus de satisfactions aux expérimentateurs, tant par la qualité de son action radioprotectrice que par sa faible toxicité.

2° Pour mettre en évidence cette radioprotection, différents tests ont été utilisés: courbes de mortalité (rats, souris, cobayes, infusoires), courbes de croissance des racines de pois, modifications histologiques au niveau des ovaires, de la muqueuse des intestins grêles, de la rate, du foie et du thymus, maturation des hématies jeunes, régénération des leucocytes, chute de poids.

3° Tandis que Gerebtzoff et Bacq[2] pensent que le mécanisme intime de la radioprotection chimique consiste surtout en une accélération et une activation de la régénération cellulaire, Desaive[3] a pu montrer qu'en plus de ce facteur, il faut aussi tenir compte d'un effet radioprotecteur direct.

4° D'autre part, R. et J. Graham[4] ainsi qu'à leur suite Kohn[5], Nielsen[6] et d'autres ont mis en évidence dans les cellules vaginales normales de patientes atteintes de cancer du col et soignées par radiothérapie, des lésions anatomiques dues aux radiations; l'importance de ces lésions serait en rapport avec la plus ou moins grande radiosensibilité de la tumeur.

Laissant de côté pour l'instant leur importance pronostique, nous nous sommes demandés si ces lésions ne pourraient pas servir de test à la fois aisé et fidèle pour mettre en évidence l'effet radio-protecteur d'une substance donnée. C'est là le but de ce travail.

II. – *Technique.* Tous les frottis vaginaux sont colorés par la méthode trichromasique de Papanicolaou. Dans chaque frottis, nous avons compté dans la mesure du possible 100 cellules intactes et nous avons déterminé le pourcentage de cellules lésées par les radiations. Pour des raisons qui apparaissent clairement dans la suite de l'exposé, nous n'avons fait entrer en ligne de compte que des cellules nucléées.

Par ailleurs, les animaux d'expérience furent des rates vierges de l'élevage sélectionné du Centre Anticancéreux, chacune pesant 150 à 175 g au départ.

III. – *Méthode.* Nous avons utilisé une irradiation intravaginale au moyen d'un tube de 50 mg de radium, dont la longueur active est de 12 mm et la filtration de 1 mm Pt; la durée d'irradiation est de 4 h. Dans ces conditions, la dose reçu à 0,5 cm de Ra serait de 4000 r γ (dose calculée d'après la méthode de Manchester).

Nous avons subdivisé notre groupe d'animaux en deux lots.

1° Un premier lot de 12 rates a subi l'irradiation dans les conditions décrites ci-dessus sous anesthésie au dial injecté par la voie intra-péritonéale.

2° Il en fut de même pour les 13 animaux du second lot mais ceux-ci reçurent immédiatement avant l'irradiation, 15 mg de Becaptan par la voie intra-péritonéale. Un frottis a été prélevé chaque jour chez chaque animal.

IV. – *Résultats. a) Lésions observées.* A notre connaissance R. et J. Graham ont été les premiers à décrire les modifications observées après radiothérapie dans les cellules vaginales de la femme; elles sont de quatre ordres:

1° vacuolisation du cytoplasme;
2° augmentation de la taille cellulaire;
3° modifications nucléaires;
4° doubles noyaux.

[1] Z. M. Bacq et P. Alexander, *Principes de Radiobiologie* (Sciences et Lettres, Liège 1955).

[2] M. A. Gerebtzoff et Z. M. Bacq, Exper. *10*, 341 (1954).

[3] P. Desaive et J. Varetto-Denoel, Exper. *11*, 242 (1955).

[4] R. M. Graham, Surg. Gynec. et Obst. *84*, 153 (1947); *93*, 767 (1951). – J. B. Graham et R. M. Graham, Cancer *6*, 68 (1953); *6*, 215 (1953).

[5] G. Kohn, Acta radiol. *12*, 446 (1954).

[6] A. M. Nielsen, Acta radiol. *36*, 479 (1952).

[7] W. J. Meredith, *Radium dosage; the Manchester System* (The Williams and Wilkin Co., 1949).

Tableau I

Pourcentage des lésions cellulaires observées dans les jours qui suivent l'irradiation des rates non protégées
(– = frottis illisible ou manquant, C = œstrus, + = sacrifice de l'animal)

Rats Jours	A	B	C	D	E	F	G	H	I	J	K	L
1	0	6	–	C	5	0	–	0	–	–	–	–
2	6	4	0	–	0	–	0	–	C	–	0	5
3	10	0	C	–	–	–	–	8	C	C	10	10
4	12	0	5	10	C	–	–	5	34	34	23	–
5	32	C	16	20	C	–	12	13	44	68	–	22
6	56	C	25	44	–	60	60	20	41	52	34	32
7	60	54	65	68	70	–	–	36	42	50	48	45
8	50	33	–	–	–	–	–	50	50	50	52	30
9	33	–	50	–	50	–	–	68	38	21	72	45
10	–	–	–	–	–	30	–	24	36	15	39	–
11	–	–	–	20	–	27	40	10	30	–	16	–
12	–	–	–	–	–	–	–	15	24	–	–	–
13	–	–	+	+	+	–	12	24	25	10	10	–
14	–	–				20	10		–	–	5	–
15	–	–				15	C		10	–		–
16	–	–				10	8		10	–		20
17	–	–				–	5		12	–		–
18	–	–				–	0					–
19	+	+				–	–					–
20						–	–					12
21						–	–					
22						C	5					
23						+	+					
24												
25												
26												
27												

Tableau II

Pourcentage des lésions cellulaires observées dans les jours qui suivent l'irradiation des rates protégées

Rats Jours	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1	2	3	2	–	0	–	–	–	C	8	C	C	C
2	0	C	C	C	–	5	–	–	10	C	C	C	5
3	2	C	0	C	5	C	0	–	–	–	C	10	0
4	0	–	–	–	C	10	5	C	–	–	16	10	18
5	–	–	8	–	–	–	–	–	12	C	20	C	18
6	8	16	20	30	30	20	–	–	5	C	34	–	32
7	20	23	25	–	–	30	26	24	20	C	20	28	18
8	20	–	–	30	–	–	–	–	25	C	10	–	23
9	–	6	–	–	–	20	–	–	–	–	–	–	–
10	–	–	–	–	20	20	–	10	21	25	–	–	16
11	8	C	–	–	–	–	–	–	–	8	–	0	–
12	–	C	10	–	25	–	–	C	5	–	12	–	6
13	0	–	–	–	–	10	–	5	C	–	10	–	C
14	–	–	–	+	10	20	5	0	–	–	–	–	–
15	+	+	+		10	–	0	–	0	C	–	–	–
16					–	–	5	–	12	0	5	–	–
17					–	C	0	–	–	–	–	9	C
18					C	15	–	–	C	3	0	5	4
19					–	–	–	–	–	+	5	–	8
20					10	–	–	–	9		–	+	+
21					–	–	–	–	+		+		
22					–	–	–	–					
23					–	–	+	+					
24					–	–							
25					–	–							
26					–	–							
27					+	+							

Dans le présent travail, nous n'avons pu tenir compte des modifications nucléaires car nous pensons que leur appréciation est fort subjective chez la rate. De plus, les cellules vaginales du rat pouvant présenter spontanément de nombreuses vacuoles, nous n'avons considéré comme élément de radio-lésions que les vacuoles volumineuses distendant et boursouflant les cellules. Nous avons surtout tenu compte de la présence de noyaux multiples et des cellules nucléées. Dans ces conditions, on peut considérer qu'un minimum de 10% de cellules anormales est indispensable pour pouvoir affirmer la présence de radio-lésions. En effet, un pourcentage variable, mais ne dépassant pas 10% de cellules normales peuvent apparaître spontanément dans les frottis vaginaux de la rate.

Les microphotographies ci-dessous montrent les lésions observées après radiations.

Fig. 1. Frottis vaginal de Rate normale – témoin (Gross. 580 ×).

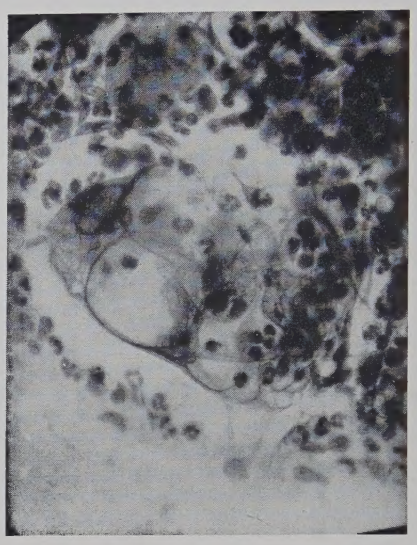


Fig. 3. Autre aspect du même frottis: en plus des caractères repris plus haut, on note l'abondance des vacuoles qui boursouflent la cellule. (Gross. 580 ×.)

Fig. 2. Frottis vaginal pratiqué sur le même animal, 6 jours après l'irradiation. A noter, comme radio-lésions, l'énorme augmentation de volume des cellules vaginales et la présence de pus (infection surajoutée). (Gross. 580 ×.)

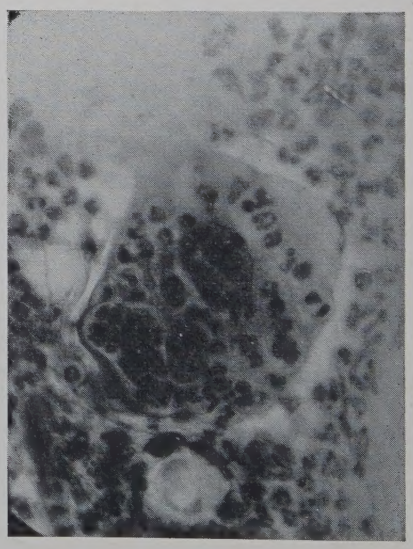


Fig. 4. Autre aspect du même frottis (Gross. 580 ×).

de l'augmentation de la taille cellulaire; nous pensons que toute cellule dont le plus grand diamètre dépasse 45μ peut être considérée comme anormale quant à la taille. Ces considérations expliquent que dans l'évaluation de nos pourcentages, nous n'avons tenu compte que

b) *Résultats.* Voici, exprimé en pourcent, le nombre de cellules radio-lésées observées le jour après l'irradiation chez chacun de nos animaux non protégés (Tableau I).

Le Tableau II donne, exprimé en %, le nombre de cellules radio-lésées observées le jour après l'irradiation chez les animaux non protégés.

V. – *Conclusions.* 1° Les radiolésions apparaissent dans les cellules vaginales de la rate le 4e ou le 5e jour qui suit l'irradiation (plutôt le 5e ou le 6e jour dans la série protégée par le Becaptan) pour atteindre leur maximum de fréquence du 5e au 9e jour (dans la série avec Becaptan, il semble que ce maximum se situe du 6e au 10e jour et puisse se maintenir pendant plusieurs jours), et redescendre petit à petit aux valeurs normales.

2° L'effet radio-protecteur du Becaptan apparaît de façon indubitable. Le pourcentage de cellules lésées oscille entre 50 et 70% chez les animaux non protégés (une seule fois, il ne dépasse pas 45%), tandis qu'il se maintient entre 20 et 34% chez les animaux protégés par la cystéamine.

3° On sait que la radiosensibilité des cellules vaginales de la femme est fort variable d'un sujet à l'autre. Par contre, la constance des réponses cellulaires aux radiations que nous avons pu observer chez la rate est remarquable; elle est probablement en rapport avec la pureté de la race des animaux employés.

4° L'importante différence entre les témoins irradiés et les protégés plaide en faveur de l'existence d'un effet radioprotecteur direct de la cystéamine.

L. Darcis[1], P. Hotterbeex et C. Onkelinx

Laboratoire de Pathologie Chirurgicale Générale et Centre Anticancéreux de l'Université de Liège, le 25 janvier 1956.

Summary

The authors studied the lesions observed in the vaginal smears of the rat after a local irradiation.

They observed that an injection of cysteamine before the irradiation strongly diminishes the ratio of damaged cells.

[1] L. Darcis, Aspirant du F. N. R. S.

Untersuchungen über einen mikrobiologisch feststellbaren Serumfaktor nach Gelbsucht

In einer früheren Arbeit haben wir über die Beeinflussung der Keimung von Pilzsporen und Blütenstaubkörnern durch menschliches Serum von Gesunden und Malignompatienten berichtet[1]. In Tabelle I sind die Ergebnisse von weiteren Keimungsversuchen mit Serum gesunder Blutspender an Sporen von *Helminthosporium sativum* und *Alternaria tenuis* und an Blütenstaub aus kurz- und langgriffligen Blüten von *Primula obconica* zusammengestellt.

Aus Tabelle I ist zu ersehen, dass Serum von gesunden Spendern die Sporenkeimung von *Helminthosporium sativum* nicht beeinflusst; bei *Alternaria tenuis* und *Primula obconica*, Langgriffel, tritt eine schwache Förderung, bei Blütenstaub von *Primula obconica*, Kurzgriffel, eine deutliche Hemmung auf.

Untersucht man Serum von Spendern, welche früher eine Gelbsucht durchgemacht haben, so stellt man fest,

[1] F. H. Schwarzenbach, Oncologia *8*, 93 (1955).

Tabelle I

Beeinflussung der Sporen- und Pollenkeimung durch Serum gesunder Blutspender

Organismus	n	V	±	s
Helminthosporium sativum . .	252	100,4	±	10,5
Alternaria tenuis	252	107,4	±	9,1
Primula obconica Kurzgriffel .	252	75,3	±	10,7
Primula obconica Langgriffel .	252	111,5	±	8,7

n = Anzahl der untersuchten Spender

$V = \frac{100 \cdot X_{\text{Serum}}}{X_{\text{Kontrolle}}}$

X_{Serum} = Keimrate bei Zusatz von Serum; bestimmt als arithmetisches Mittel aus einer Stichprobengruppe von 5 × 50 Sporen oder Pollenkörnern.

$X_{\text{Kontrolle}}$ = Keimrate in der Kontrolle ohne Zusatz von Serum; bestimmt als arithmetisches Mittel aus einer Stichprobengruppe von 10 × 50 Sporen oder Pollenkörnern.

s = Standardabweichung

dass in vielen Fällen die Verhältniszahlen V unter den entsprechenden Werten für gesunde Spender liegen.

In Tabelle II sind zur Illustration zwei Serien gleichzeitig untersuchter Blutproben einander gegenübergestellt; eine erste Gruppe umfasst Seren von 16 gesunden Spendern. Die Proben der zweiten Serie stammen von Personen, welche entweder an Gelbsucht erkrankt waren oder mit Gelbsuchtpatienten in Kontakt gestanden hatten.

Die Mittelwerte für Spender mit positiver Gelbsuchtanamnese liegen tiefer als die Durchschnitte in der Gruppe der Spender, die nie an Gelbsucht erkrankt waren. Nach dem Trennverfahren von R. A. Fisher[2] wurde für die beiden Gruppen A und B die folgende Trennformel berechnet:

$$Z = X_1 + 0{,}35 \cdot X_2 + 0{,}83 \cdot X_3 + 0{,}05 \cdot X_4$$

In dieser Formel bedeuten:

X_1 = Verhältniszahl für *Helminthosporium sativum*;
X_2 = Verhältniszahl für *Alternaria tenuis*;
X_3 = Verhältniszahl für *Primula obconica*, Kurzgriffel;
X_4 = Verhältniszahl für *Primula obconica*, Langgriffel*.

* In der untersuchten Serie keimte der Langgriffelpollen von *Primula obc.* sehr ungleichmässig, was den niedrigen Koeffizienten von 0,05 erklärt. Obwohl der Langgriffelpollen deshalb wenig zur Trennung der beiden Gruppen beiträgt, wurde X_4 zur Illustration des Verfahrens mit in die Trennformel einbezogen.

Für jede Blutprobe wurde nach dieser Formel der Wert von Z berechnet (siehe Tab. II). Der Mittelwert von Z für die Gruppe B (Gelbsuchtproben) liegt mit 188 Einheiten deutlich unter dem Durchschnitt $Z = 214$ der Gruppe A (gesunde Spender). Der Unterschied ist nach dem F-Test statistisch mit einer Wahrscheinlichkeit $P < 0{,}001$ stark gesichert. Der berechnete Wert von F ist mit 6,67 grösser als die einem $P = 0{,}001$ entsprechende Zahl 6,43. (Freiheitsgrade $n_1 = 4$, $n_2 = 25$.)

[2] Vgl. A. Linder, *Statistische Methoden für Naturwissenschafter, Ärzte und Ingenieure*, 2. Aufl. (Birkhäuser, Basel 1951), S. 65.

Tabelle II. Beeinflussung der Sporen- und Pollenkeimung durch Serum von Spendern mit negativer und positiver Gelbsuchtanamnese

A. Spender mit negativer Gelbsuchtanamnese

Nr.	Verhältniszahlen für					
	Helminthosporium sativum	*Alternaria tenuis*	*Primula obconica* Kurzgriffel	*Primula obconica* Langgriffel	*Z*	Reaktion
1	96	103	100	107	220	–
2	101	84	96	84	207	–
3	91	101	87	80	203	–
4	96	110	102	105	224	–
5	109	107	89	71	224	–
6	101	106	90	110	218	–
7	103	108	84	120	217	–
8	103	108	78	120	212	–
9	107	92	80	92	210	–
10	108	103	76	108	213	–
11	110	101	81	91	217	–
12	99	118	84	114	216	–
13	110	106	78	113	218	–
14	104	104	80	110	212	–
15	108	104	74	108	209	–
16	103	105	80	118	212	–
Mittelwerte	103	104	85	103	214	

B. Spender mit positiver Gelbsuchtanamnese

Nr.	Verhältniszahlen für						
	Krankheitsjahr	*Helminthosporium sativum*	*Alternaria tenuis*	*Primula obconica* Kurzgriffel	*Primula obconica* Langgriffel	*Z*	Reaktion
17	1918	98	103	72	114	200	+
18	1918	102	112	66	104	202	+
19	1924	92	85	71	114	187	++
20	1931	93	108	76	114	201	+
21	1939	100	79	57	94	180	++
22	1940	97	83	64	86	183	++
23	1942	77	101	71	67	174	++
24	1943	84	93	53	74	164	++
25	1943	89	83	62	92	174	++
26	1943	86	84	69	94	177	++
27	1943	110	96	73	81	208	–
28	1945	68	102	93	115	187	++
29	1953	76	93	83	79	183	++
30	1955	96	109	95	103	217	–
Mittelwerte		91	95	72	95	188	

Reaktion – negativ, + schwach positiv, ++ eindeutig positiv *V*: siehe Tabelle I *Z*: Erklärung im Text.

Tabelle III. Häufigkeit positiver Gelbsuchtreaktion bei Spendern mit negativer und positiver Gelbsuchtanamnese

	n	*p*	%	Vertrauensgrenzen bei einem $P = 0{,}01$	
Spender, die gemäss ihren eigenen Angaben nie an Gelbsucht erkrankt waren	344	5	1,5	untere Grenze 0,4%	obere Grenze 3,8%
Spender, die an Gelbsucht erkrankt waren	50	36	72,0	54 % (abgerundet)	87 % (aufgerundet)

n = Anzahl der untersuchten Blutproben
p = Anzahl der positiv reagierenden Blutproben
% = Prozentsatz der positiv reagierenden Blutproben

Die Vertrauensgrenzen wurden für ein $P = 0{,}01$ nach den bei A. Linder[3] angegebenen Verfahren berechnet.

[3] A. Linder, *Statistische Methoden für Naturwissenschafter, Ärzte und Ingenieure*, 2. Aufl. (Birkhäuser, Basel 1951), S. 118.

Wie Tabelle II zeigt, reagieren in Gruppe *B* die beiden Spender Nr. 27 und 30 negativ. Im ersten Fall (Nr. 27) fehlen genauere Angaben; im zweiten Fall (Nr. 30) handelt es sich um eine Spenderin, welche an einer leichten anikterischen Hepatitis erkrankt war. Die Proben Nr. 17 ($Z = 200$) dafür, Nr. 18 ($Z = 202$) und Nr. 20 ($Z = 201$) weisen eine nur schwach positive Reaktion auf. Alle drei Spender machten vor mehr als 20 Jahren eine Gelbsucht durch. Es wäre jedoch verfrüht, diese Beobachtung dahin zu verallgemeinern, dass weit zurückliegende Erkrankungen an Gelbsucht schwach positiv oder negativ reagieren. Weitere Untersuchungen haben ergeben, dass selbst Spender, die 1905 bzw. 1916 an Gelbsucht erkrankten, positiv reagierten.

Um festzustellen, wie häufig die unspezifische Keimungshemmung unter Spendern mit positiver oder negativer Gelbsuchtanamnese auftritt, wurden zwei weitere Versuchsreihen von 50 bzw. 344 Proben untersucht.

Die Versuche ergeben, dass positive Gelbsuchtreaktionen vorwiegend auf die Gruppe derjenigen Spender beschränkt sind, die früher an Gelbsucht erkrankt waren. Von den positiv reagierenden Spendern unter den Gesunden konnten 2 nachkontrolliert werden. Der erste Proband hatte eine Gelbsucht durchgemacht, ohne ärztliche Behandlung in Anspruch zu nehmen; der zweite war nie an Gelbsucht erkrankt.

Tabelle IV. Beeinflussung der Sporen- und Pollenkeimung durch Serum bei akuter *Hepatitis epidemica*

Organismus	n	$V \pm s$	d
Helminthosporium sativum . .	6	93,8 ± 3,6	– 6,6
Alternaria tenuis	6	92,5 ± 5,2	–14,9
Primula obconica Kurzgriffel .	6	74,8 ± 5,3	– 0,5
Primula obconica Langgriffel .	6	92,9 ± 4,8	–18,6

n, V, s siehe Tabelle I
d Differenz der Mittelwerte für gesunde und gelbsüchtige Spender

Tabelle IV zeigt, dass alle sechs untersuchten Fälle von akuter *Hepatitis epidemica* eindeutig positiv reagierten.

Die Keimungshemmung durch Serum von Gelbsuchtkranken wirkt sich vor allem auf den Langgriffelpollen von *Primula obconica* und auf die Sporen von *Alternaria tenuis* aus. Der Kurzgriffelpollen von *Primula obconica* wird am wenigsten gehemmt.

Bei anderen Krankheiten mit negativer Gelbsuchtanamnese tritt die Keimungshemmung nicht auf. Unter 102 Fällen bei 58 verschiedenen Krankheiten liess sich nur ein einziges Mal bei einem Diabetiker eine positive Gelbsuchtreaktion nachweisen. Bei Malignompatienten ist der Keimungstest gestört. In zwei Fällen von tödlich ausgegangener, posttransfusioneller Hepatitis konnte das Blut der in Frage kommenden Spender untersucht werden. In beiden Fällen wurde je ein positiver Reagent mit negativer Gelbsuchtanamnese aufgefunden.

Die Versuche über diese mikrobiologische Serumreaktion werden am Zentrallaboratorium des Blutspendedienstes des Schweizerischen Roten Kreuzes in Bern fortgesetzt.

Die Arbeit wurde durch den Schweizerischen Nationalfonds für wissenschaftliche Forschung unterstützt.

F. H. Schwarzenbach

Zentrallaboratorium des Blutspendedienstes des Schweizerischen Roten Kreuzes, Bern, den 10. März 1956.

Summary

The germination of spores and pollen grains is often inhibited by the serum of subjects who have once suffered from a jaundice. Experiments have been done with spores of *Helminthosporium sativum* and *Alternaria tenuis* and with pollen grains of short style and long style plants of *Primula obconica*. 36 samples of a series of 50 persons, who have suffered from a jaundice, showed the inhibition of germination. 5 samples with positive reaction have been found in a second series of 344 sera of subjects who have never suffered from a jaundice. We observed positive reaction with *Hepatitis epidemica acuta* (6 samples); all other kind of diseases so far tested did not show any similar reaction.

Effect of Vitamins on the Acute Toxicity of Hydrazine Derivatives*

Although iso-nicotinic acid hydrazide (INH) is much used in the chemotherapy of tuberculosis, it is recognized that it has a central stimulating effect and in higher dosages produces convulsions[1]. Death due to INH-therapy in epileptics have been reported[2]. To some extent the central stimulation of INH could be controlled by barbiturates[3] and also by pyridoxin[4]. Although pyridoxin does not diminish the antitubercular activity of INH[5], it gives protection against the peripheral neuritis caused by the drug under chronic administration[6].

In view of the success of INH in the chemotherapy of tuberculosis, other hydrazine-derivatives have been prepared and examined for antitubercular activity. Among these, recently, cyanacetic acid hydrazide (CAH) has come into clinical use[7].

Since pyridoxin influences the toxicity of INH, the effects of this and other vitamins of the B-group on the activity of some hydrazine compounds were investigated.

INH, CAH, phenylhydrazine-HCl and hydrazine-HCl, dissolved in water, were given intraperitoneally to guinea pigs at the dosages indicated. The vitamins were injected simultaneously by the same route. The animals were then observed for a period of 20 h for signs of toxicity. The results are presented in the Figure and Tables I and II.

From the results it may be seen that, among the vitamins tested, only pyridoxin possessed significant activity over the acute toxicity of INH and CAH in

* Grateful acknowledgements are made to Hoffmann-La Roche Ltd., Basle, for gifts of INH (Rimifon) and vitamins, and to Laboratoires OM, Geneva, for CAH (Reazide).

[1] W. M. Benson, P. L. Stefko, and M. D. Roe, Amer. Rev. Tuberc. *65*, 376 (1952).

[2] K. J. Fetterhoff, C. X. Holmes, and G. E. Martin, Amer. Rev. Tuberc. *66*, 501 (1952).

[3] S. Y. P'an, L. Markaroglu, and J. Reilly, Amer. Rev. Tuberc. *66*, 100 (1952).

[4] R. H. Reilly, K. F. Killam, E. H. Jenney, W. H. Marshall, T. Tausig, N. S. Apter, and C. C. Pfeiffer, J. Amer. med. Assoc. *152*, 1317 (1953). – B. Fust, *Bericht über das 2. Rimifon-Kolloquium in Oestrich (Deutschland)*, 18. April 1953.

[5] E. Grunberg and W. Blencowe, Amer. Rev. Tuberc. *71*, 898 (1955).

[6] J. P. Biehl and H. J. Nimitz, Amer. Rev. Tuberc. *70*, 430 (1954).–Editorial, Brit. med. J. *1955*, 1203. – G. Zbinden and A. Studer, Schweiz. Z. Path. Bakt. *18*, 1198 (1955).

[7] H. Scheu, Schweiz. Z. Tuberk. *11*, 77 (1954).

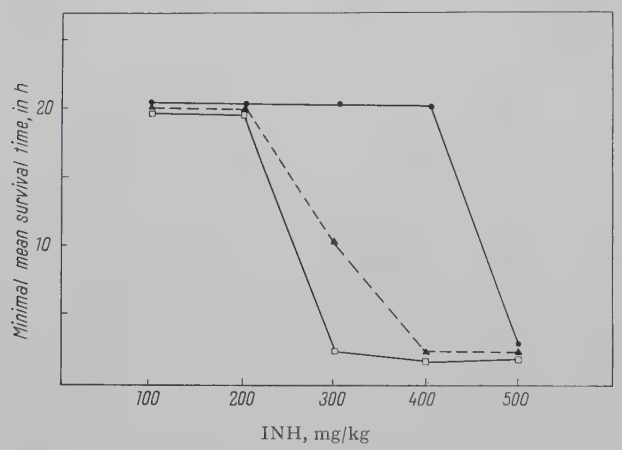
Table I

Effect of some B-Vitamins on the Acute Toxicity of Cyanacetic Acid Hydrazide in Guinea Pigs

Cyanacetic acid hydrazide mg/kg	Minimal mean survival time, in h						
	no vitamin	pyridoxin 300 mg/kg	thiamin 300 mg/kg	niacin 300 mg/kg	panthenol 300 mg/kg	folic acid 50 mg/kg	riboflavin 20 mg/kg
100	20·0 (3)	20·0 (1)	20·0 (1)	20·0 (1)	20·0 (1)	20·0 (1)	20·0 (1)
150	11·0 (1)	20·0 (1)	–	20·0 (1)	–	–	–
200	8·0 (7)	20·0 (4)	15·0 (2)	3·8 (1)	20·0 (1)	20·0 (1)	2·0 (1)
250	5·0 (3)	20·0 (3)	–	3·0 (1)	–	–	–
300	4·3 (8)	16·8 (5)	4·5 (2)	3·0 (1)	1·5 (1)	2·3 (1)	2·0 (1)
400	2·7 (10)	10·0 (7)	5·0 (2)	5·3 (1)	2·0 (1)	2·5 (1)	1·5 (1)
500	2·1 (7)	11·3 (4)	2·5 (2)	2·5 (3)	1·5 (1)	1·8 (1)	2·0 (1)
600	2·2 (4)	6·3 (3)	2·0 (1)	–	–	–	–
800	1·5 (1)	3·5 (1)	–	–	–	–	–

Vitamins were administered simultaneously with CAH by intraperitoneal injection. Figures in ( ) indicate the number of animals used.

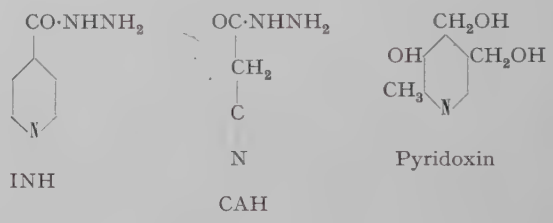
guinea pigs. Since pyridoxin and INH have a somewhat similar structure, it is likely that INH interferes with pyridoxin-metabolism in the guinea pig. As the toxicity



With Pyridoxin, 300 mg/kg; with Thiamin, 300 mg/kg; INH alone

Effect of Pyridoxin and Thiamin on the Acute Toxicity of INH in Guinea Pigs

of CAH is also influenced by pyridoxin, it would appear that CAH acts in a manner similar to INH. Biehl and Vilter[8] suggested that INH does not compete with pyridoxin, but that it depletes the tissues of pyridoxin by forming a hydrazone which is excreted. It is likely that CAH also binds pyridoxin.



The results with phenylhydrazine-HCl and hydrazine-HCl, presented in Table II, indicate that pyridoxin does not bear a demonstrable relation to the toxicity of these compounds. Further, INH and CAH given in toxic amounts induced several typical attacks of clonic convulsions, and with lethal doses the animals died apparently as a result of respiratory failure with tonic extension of legs. However, these signs of stimulation of the central nervous system were not observed with phenylhydrazine and hydrazine. It would, therefore, appear that the toxic action of hydrazines is different from that of acid-hydrazides like INH and CAH.

M. O. Tirunarayanan and
W. A. Vischer

Schweizerisches Forschungsinstitut für Tuberkulose, Davos-Platz, April 9, 1956.

Table II

Effect of Pyridoxin on the Acute Toxicity of Hydrazine and Phenylhydrazine in Guinea Pigs

mg/kg	Minimal mean survival time, in h			
	hydrazine-HCl		phenylhydrazine-HCl	
	no vitamin	pyridoxin	no vitamin	pyridoxin
50	20·0	20·0	–	–
100	15·0	15·0	20·0	20·0
150	4·5	15·0	–	–
200	3·75	3·75	12·0	12·0
300	3·0	3·75	1·5	4·5
400	–	–	0·75	0·95
500	–	–	0·75	0·75

Pyridoxin administered simultaneously with the drugs at 300 mg/kg; 5 animals were used for each group.

Zusammenfassung

Einige Vitamine der B-Gruppe wurden auf ihre Fähigkeit geprüft, die akute Toxizität von Hydrazinderivaten beim Meerschweinchen herabzusetzen. Nur Pyridoxin zeigte eine schützende Wirkung gegen die Toxizität von Säurehydraziden wie Isonikotinsäurehydrazid und Cyanessigsäurehydrazid, nicht aber gegen diejenige von Hydrazin und Phenylhydrazin.

[8] J. P. Biehl and R. W. Vilter, Proc. Soc. exper. Biol. Med. *85*, 389 (1954).

Mydriatic Activity of Analgesics in Mice

Although morphine is known to produce mydriasis in mice, the mydriatic activity of morphine-like drugs has received only little attention in the past. Its mechanism seems to be unknown.

In the course of a screening program involving the investigation of a new series of synthetic analgesics and parasympatholytics, we found a highly significant positive correlation between the analgesic and the mydriatic activities in mice of known and new morphine-like analgesics.

The analgesic activity in mice (ED_{50} in mg/kg subcutaneously = A) was determined using the "hot plate" technique, described by EDDY *et al.*[1].

The mydriatic activity in mice (ED_{50} in mg/kg subcutaneously = M) was determined using essentially the same method as described in previous papers[2].

The results obtained with 20 known analgesics of various structure are summarized in the Table, and graphically represented in the Figure.

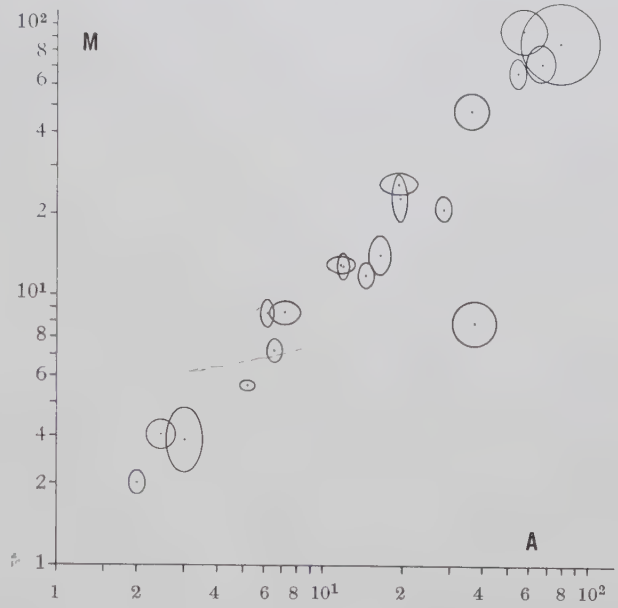
The rank correlation coefficient τ for the two rankings of A and M is highly significant

$$(\tau = +0{\cdot}79;\ \frac{S-1}{\sigma} = \frac{149}{30{\cdot}8} = 4{\cdot}83;\ P < 0{\cdot}01)\ .$$

[1] N. B. EDDY, C. F. TOUCHBERRY, and J. E. LIEBERMAN, J. Pharmacol. exper. Therap. *98*, 121 (1950). – N. B. EDDY and D. LEIMBACH, J. Pharmacol. exper. Therap. *107*, 385 (1953). – D. LEIMBACH and N. B. EDDY, J. Pharmacol. exper. Therap. *110*, 135 (1954).

[2] D. K. DE JONGH, E. G. VAN PROOSDIJ-HARTZEMA, and P. JANSSEN, Arch. int. Pharmacodyn. *103*, 100 (1955). – E. G. VAN PROOSDIJ-HARTZEMA, P. JANSSEN, and D. K. DE JONGH, Arch. int. Pharmacodyn. *103*, 120 (1955). – A. JAGENEAU and P. JANSSEN, Arch. int. Pharmacodyn. *106*, 199 (1956).

The relatively high A/M ratio for the ethyl ester No. 16 [A/M = 4·57 (3·60–5·80)] is probably due to the fact



Analgesic and mydriatic activity of 20 morphine-like analgesics in mice

A analgesia (ED_{50} in mg/kg subcutaneously)
M mydriasis (ED_{50} in mg/kg subcutaneously)

that this compound is a more active parasympatholytic than the other compounds listed. As an acetylcholine-

No.		Number of mice used	ED_{50} in mg/kg subcutaneously and fiducial limits (P 0·05)		A/M	Rankings A	Rankings M
1	Heroine hydrochloride	185	2·00 (1·84– 2·18)	2·03 (1·83– 2·25)	0·99	1	1
2	*dl*-Phenadoxone HBr	115	2·45 (2·15– 2·79)	3·08 (2·77– 3·42)	0·80	2	3
3	1-(3-Hydroxy-N-methyl)-morphinane *d*-tartrate	110	3·00 (2·56– 3·51)	2·94 (2·21– 3·91)	1·02	3	2
4	*dl*-Amidone HCl	210	5·18 (4·80– 5·59)	4·75 (4·52– 4·99)	1·09	4	4
5	4:4-Diphenyl-6-(N-morpholino)-hexan-3-one base	105	6·15 (5·77– 6·55)	8·75 (7·74– 9·89)	0·70	5	7
6	3-(N-piperidino)-1:1-di-2′-thienyl-prop-1-ene HCl	115	6·50 (5·99– 7·05)	6·40 (5·82– 7·04)	1·02	6	5
7	*dl*, 4:4-Diphenyl-5-methyl-6-(N-morpholino)-3-acetylimino-hexane HCl	175	7·10 (6·17– 8·17)	8·90 (7·95– 9·97)	0·80	7	8
8	4:4-Diphenyl-6-dimethylamino-hexan-3-one HCl	110	11· 6 (10· 3– 13· 1)	13· 2 (12· 3– 14· 1)	0·88	8	11
9	Morphine hydrochloride	345	11· 8 (11· 2– 12· 5)	13· 1 (11· 7– 14· 7)	0·90	9	10
10	4:4-Diphenyl-6-(N-pyrrolidino)-hexan-3-one base	110	14· 3 (13· 1– 15· 9)	12· 2 (11· 0– 13· 5)	1·17	10	9
11	4:4-Diphenyl-6-(N-piperidino)-hexan-3-one HCl	140	16· 2 (14· 5– 18· 1)	14· 5 (12· 3– 17· 1)	1·12	11	12
12	*dl*, 4:4-Diphenyl-5-methyl-6-(N-morpholino)-3-iminohexane HCl	155	19· 0 (16· 1– 22· 4)	26· 6 (24· 2– 29· 3)	0·71	12	15
13	Ethyl, 2:2-diphenyl-4-(N-morpholino)-butyrate base	65	19· 2 (17· 9– 20· 5)	23· 7 (19· 3– 29· 2)	0·81	13	14
14	Pethidine HCl	160	28· 0 (25· 7– 30· 5)	21· 5 (19· 5– 23· 7)	1·30	14	13
15	3:3-Diphenyl-5-(N-morpholino)-pentan-3-one HCl	80	35· 8 (31· 1– 41· 2)	50· 5 (43· 2– 59· 1)	0·71	15	16
16	Ethyl, 2:2-diphenyl-4-(N-piperidino)-butyrate oxalate	100	37· 0 (31· 4– 43· 7)	8·10 (6·75– 9·72)	4·57	16	6
17	Codeine phosphate	135	53· 0 (48· 2– 58· 3)	70· 0 (61· 4– 79· 8)	0·76	17	17
18	Dionine hydrochloride	85	55· 8 (45· 7– 68· 1)	100· 0 (82· 0–122· 0)	0·56	18	20
19	Alpha, *dl*, 4-dimethylamino-3-methyl-2-propionyloxybutane HCl	60	65· 8 (57· 7– 75· 0)	76· 0 (64· 4– 89· 7)	0·87	19	18
20	*dl*-4:4-Diphenyl-5-methyl-6-(N-morpholino)-hexan-3-one base	70	77· 5 (53· 8–111· 6)	90· 5 (65· 6–124· 9)	0·86	20	19

antagonist on the isolated rabbit ileum compound, No.16 is 1·44% (1·07–1·94%) as active as atropine sulfate. The other compounds listed are less active or inactive in these experimental conditions[3]. E.g. amidone: 0·50 (0·37–0·68%); pethidine: 0·30 (0·22–0·41%); morphine: < 0·1%.

Investigation into various aspects of this problem is being pursued; the results will be published in detail elsewhere.

Acknowledgment. The authors wish to express their gratitude to A. F. Green (Beckenham) and A. Pohland (Indianapolis) for samples of the compounds No. 6 and No. 19 resp., and to J. Huygens and R. Frederickx for their splendid technical assistance.

P. A. J. Janssen and A. Jageneau

Research Laboratory Eupharma, Pharmaceutical Laboratories Dr. J. C. Janssen, Turnhout (Belgium), April 17, 1956.

Résumé

Dans une série de 20 analgésiques puissants nous avons trouvé une correlation hautement significative entre l'activité analgésique et mydriatique chez la souris.

[3] D. K. de Jongh, E. G. van Proosdij-Hartzema, and P. Janssen, Arch. int. Pharmacodyn. *103*, 100 (1955).– E. G. van Proosdij-Hartzema, P. Janssen, and D. K. de Jongh, Arch. int. Pharmacodyn. *103*, 120 (1955).

Glukose als Cofactor bei der Retraktion des Blutgerinnsels

Vor kurzem wurde mitgeteilt[1], dass zur Auslösung der Retraktion eines plättchenhaltigen Fibringerinnsels Thrombin und ein dialysierbarer Faktor aus Plasma oder Serum erforderlich sind.

Seither konnte festgestellt werden, dass die aktive Komponente aus Dialysat weder von Kationen- noch Anionenaustauscherharzen zurückgehalten wird. Derart entsalzte Dialysate wurden der Papierchromatographie[2] mit Butanol–Eisessig–Wasser (4:1:5) unterworfen. Die aktive Komponente konnte eluiert und als Glukose identifiziert werden.

Schon früher wurde festgestellt[3], dass die Retraktion in einem ungepufferten «Minimalsystem» aus Plättchen, Fibrinogen, Dialysat aus Plasma und Thrombin mit einem Absinken des pH-Wertes verbunden ist. Nunmehr wurde gefunden, dass die bei Glukose-Anwesenheit gebildeten Säuremengen innerhalb bestimmter Grenzen proportional dem Retraktionseffekt sind. Die gebildete Säure konnte als Milchsäure identifiziert werden, so dass offensichtlich glykolytische Prozesse im Thrombozyten auf das engste mit dem Retraktionsmechanismus verknüpft erscheinen. Die Beziehung zwischen zugesetzter Glukose und gebildeter Säure ist komplizierterer Natur; insbesondere wurde gefunden, dass mit Thrombin allein inkubierte Plättchen ebenfalls schon geringe Mengen Milchsäure zu produzieren vermögen, was auf einen natürlichen Kohlehydratgehalt der Plättchen schliessen lässt. Zur Auslösung der Retraktion genügen diese geringen plättcheneigenen Kohlehydratmengen in der Regel nicht. Immerhin mögen sie eine Erklärung dafür bieten, wieso die «viskose Metamorphose» der Plättchen oft schon beim blossen Kontakt mit Thrombin manifest wird, ohne jedoch mit dem von uns bisher benutzten Retraktions-Hemmungstest[3] fassbar zu werden.

Weitere Arbeiten zur Abklärung der Beziehungen zwischen Fermentaktivität der Plättchen und Retraktion sind im Gange.

E. F. Lüscher

Theodor-Kocher-Institut und Physiologisches Institut der Universität Bern, den 23. Juni 1956.

Summary

Dextrose is found to be a co-factor for clot retraction. During retraction lactic acid is produced, suggesting a close relationship between enzymatic activity of the platelets and the retraction-mechanism.

[1] E. F. Lüscher, Exper. im Druck (1956); Vox Sanguinis *1*, 133 (1956).
[2] S. M. Partridge, Biochem. J. *42*, 238 (1948).
[3] E. F. Lüscher, Vox Sanguinis *1*, 133 (1956).

Über die Wirkung von Phenylbutazon auf den Tricarbonsäurezyklus und andere Fermentsysteme

Über die Wirkung von Phenylbutazon (1, 2-Diphenyl-3, 5-dioxo-4-*n*-butyl-pyrazolidin = Butazolidin Geigy) auf spezifische Fermentsysteme *in vitro* liegen bis heute nur vereinzelte Angaben vor. Eine Zusammenstellung der Literatur über diesen Fragenkomplex erscheint in einer besonderen Mitteilung[1].

Unsere Versuche gingen von der Frage aus, ob die entzündungshemmende Wirkung des Phenylbutazons mit dem Stoffwechsel der Polysaccharide der Grundsubstanz verknüpft sei. *In vitro*-Versuche mit Hyaluronidase (siehe auch Gialdroni und Grassi[2]; Agolini und Mitarbeiter[3] und Domenjoz[4]) und Chondroitinase verliefen negativ (Phenylbutazonkonzentrationen von 10^{-3} *m* entsprechend 31,8 mg%). Wir untersuchten ferner den Einfluss von Phenylbutazon auf die β-Glucuronidase (Technik von Talalay und Mitarbeitern[5]) und die Cholinesterase (Methode nach Ammon[6]), ohne einen Hemmeffekt zu beobachten ($c = 10^{-3}$ *m*).

Im Hinblick auf die zentrale Stellung des Tricarbonsäuresystems im Stoffwechsel befassten wir uns ferner mit der Wirkung des Phenylbutazons auf die Zwischenstufen des Krebs'schen Zyklus.

Methoden. Die Darstellung der Mitochondrien aus Rattenleber geschah nach der Methode von Leuthardt und Müller[7]. Bei einigen Versuchen wurde diesen Ansätzen noch Glykolkomplexon (Äthylenglykol-bis-β-amino-äthyl-äther-N, N'-tetraessigsäure = schweiz. Pat.

[1] R. Pulver, B. Exer und B. Herrmann, Schweiz. med. Wschr. *86* (1956) (im Druck).
[2] G. Gialdroni und C. Grassi, Boll. Soc. Ital. Biol. sper. *28*, 1580 (1952).
[3] G. Agolini, A. Bertelli und G. Cavicchini, Boll. Soc. Ital. Biol. sper. *28*, 1761 (1952).
[4] R. Domenjoz, Arch. exp. Path. Pharm. *225*, 14 (1955).
[5] P. Talalay, W. H. Fishman und Ch. Huggins, J. biol. Chem. *166*, 757 (1946).
[6] R. Ammon, Arch. ges. Physiol. *233*, 468 (1933).
[7] F. Leuthardt und A. F. Müller, Exper. *4*, 278 (1948).

294 554) in einer Endkonzentration von 10^{-4} *m* zugesetzt (RAAFLAUB[8]).

Die Bestimmung des Sauerstoffverbrauchs erfolgte manometrisch im Warburg-Apparat bei 38° mit Luft im Gasraum.

Zur Oxydation des Pyruvats wurde das von LEUTHARDT und MAURON[9] beschriebene Versuchsmilieu verwendet. Um die Versuchsbedingungen gleichzuhalten, wurde auch zur Oxydation der anderen Substrate das gleiche Milieu gewählt. Entsprechend den vorhandenen Warburggefässen wurde das Volumen des Ansatzes auf 1,65 ml reduziert.

Der Ansatz enthielt jeweils:
0,4 ml von 0,04 *m* Phosphatpuffer, pH 7,4
0,2 ml Magnesiumsulfat 0,1 *m*
0,2 ml Kaliumchlorid 0,5 *m*
0,1 ml KCl isotonisch
0,15 ml Adenosintriphosphat 0,01 *m*
0,2 ml Mitochondriensuspension
0,25 ml Pyruvat oder andere Säuren des Tricarbonsäurezyklus 0,05 *m*.

Wasser, Phenylbutazon oder andere Zusätze wurden bis zu einem Gesamtvolumen von 1,65 ml zugegeben. Zur Absorption der Kohlensäure wurde ein gefaltetes Filterpapier (4 × 1 cm) mit 0,1 ml Natronlauge 2*n* in den Zentralzylinder gesteckt.

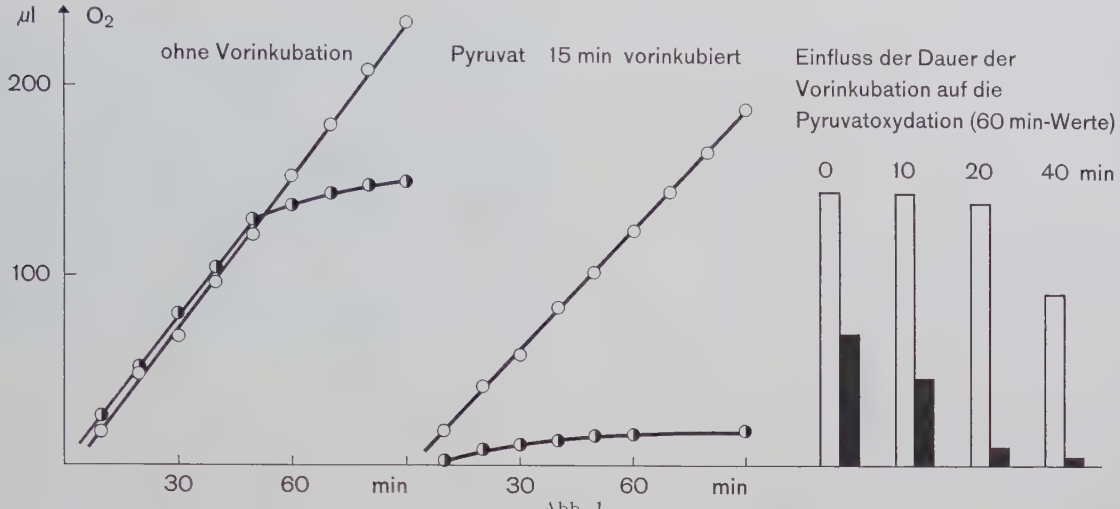


Abb. 1.

Die Versuche wurden auf zwei Arten ausgeführt. Entweder wurde der ganze Ansatz in den Hauptraum pipettiert und die erste Ablesung nach 10 min ausgeführt, oder es wurde das Substrat in den Seitenarm eingefüllt und erst nach 15 min zum Ansatz zugekippt, wobei die erste Ablesung vor dem Kippen erfolgte. Der Sauerstoffverbrauch der Kontrollen ohne Substrat betrug in 90 min etwa 30 µl und wurde in den späteren Versuchen nicht mehr bestimmt.

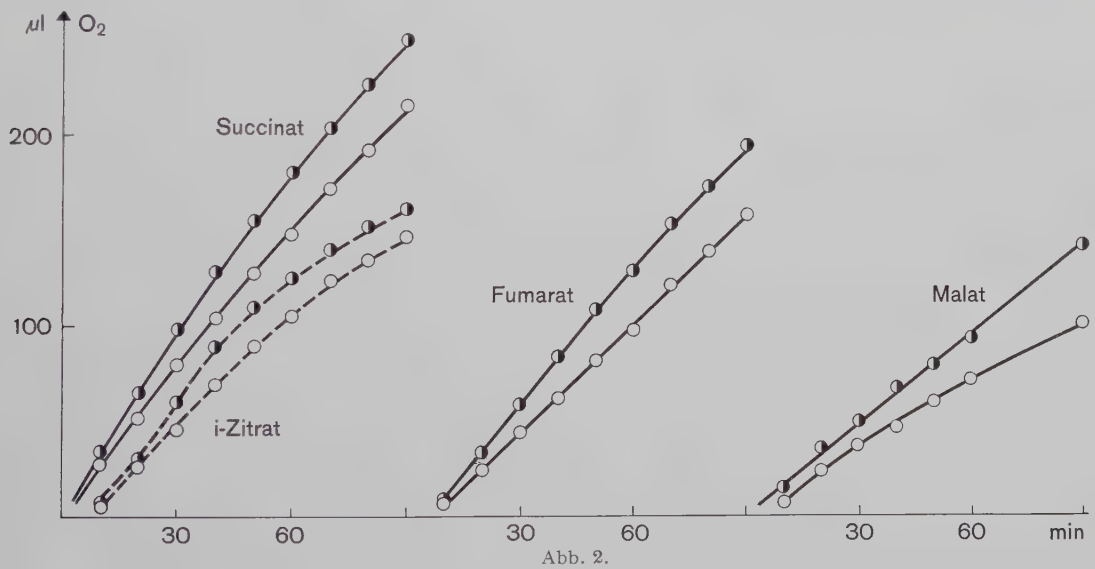


Abb. 2.

Weisse Kreise und weisse Säulen = Kontrollen; schwarz-weisse Kreise und schwarze Säulen = Phenylbutazon $3 \cdot 10^{-4}$ m.

Resultate. Phenylbutazon beeinflusst die verschiedenen Umsetzungen des Zitronensäurezyklus je nach Konzentration teils aktivierend, teils hemmend.

Die Oxydation der Brenztraubensäure (Abb. 1) wird durch Phenylbutazon (wirksame Endkonzentration

[8] J. RAAFLAUB, Helv. chim. Acta *38*, 27 (1955).
[9] F. LEUTHARDT und J. MAURON, Helv. physiol. pharmacol. Acta *8*, 386 (1950).

$3 \cdot 10^{-4}$ *m*) gehemmt. Durch Vorinkubation der Mitochondrien mit Phenylbutazon und ohne Substrat lässt sich die Hemmung der α-Ketosäurenoxydation verstärken. Eine gleichartige Behandlung der Kontrollen (ohne Phenylbutazon) schädigt bei kurzer Inkubationsdauer die Mitochondrien nur geringfügig, denn der Sauerstoffverbrauch nach Zukippen des Substrates ist nur wenig herabgesetzt (Abb. 1). Zusätze von Diphosphopyridinnukleotid ($2{,}5 \cdot 10^{-4}$ *m*), Cocarboxylase ($3{,}5 \cdot 10^{-4}$ *m*), Mn^{++} $3 \cdot 10^{-4}$ *m*, Cytochrom *c* ($4 \cdot 10^{-4}$ *m*) und Thyroxin ($8{,}6 \cdot 10^{-4}$ *m*) haben keinen Einfluss auf die Veratmung des Pyruvates. Auch nach Vorinkubation ohne Substrat lässt sich kein Einfluss dieser Faktoren feststellen. Die erwähnten Wirkstoffe können auch die Hemmung der Atmung durch Phenylbutazon nicht aufheben.

Die Oxydation der iso-Zitronensäure, Bernsteinsäure, Fumarsäure und Äpfelsäure wurde durch Phenylbutazon ($3 \cdot 10^{-4}$ *m*) nicht gehemmt. In vielen Fällen wurde der Abbau sogar beschleunigt (Abb. 2).

Die Oxydation der Zitronensäure wurde in verschiedenen Versuchsreihen teils gehemmt, teils beschleunigt, was wir der verschiedenen Herkunft der verwendeten Ratten zuschreiben möchten. Die Empfindlichkeit der einzelnen Mitochondriensuspensionen auf Phenylbutazon ($3 \cdot 10^{-4}$ *m*) war, trotz einer im Kontrollversuch gleich starken enzymatischen Aktivität, verschieden; wurde Ketoglutarat als Substrat verwendet, so konnten vorübergehende Aktivierungen, die aber bald in eine komplette Hemmung umschlugen, beobachtet werden. Versuche zeigten, dass die Dauer der initialen Aktivierung der α-Ketoglutaratoxydation bei steigender Phenylbutazonkonzentration immer kürzer wird; oberhalb 25 mg% wurden nur noch Inaktivierungen beobachtet[1]. Die zuletzt erwähnten Versuche wurden ohne Vorinkubation durchgeführt.

B. Exer

Aus den pharmakologischen Laboratorien der J. R. Geigy AG., Basel, den 30. April 1956.

Summary

Cholinesterase, hyaluronidase, chondroïtinase and β-glucuronidase are not inhibited by phenylbutazone in concentrations up to 10^{-3} *M*.

Phenylbutazone accelerates the oxidation of the acids of the Krebs cycle in therapeutic concentrations up to 10 mg%; in higher concentrations it acts as an inhibitor. The inhibition of the α-keto acid oxidation begins at lower concentrations of phenylbutazone then for the other intermediates.

Synaptic Connections of Ib Muscle Afferents to Ventral Spinocerebellar Tract Neurons

The ventral spino-cerebellar tract (v.s.c.t.) has been mapped mainly through neuro-anatomical work, and the results from a recent electrophysiological analysis[1] conform with the classical concepts. Experiments are reported here showing activation of v.s.c.t. neurons by group I*b* muscle afferents, thus suggesting exitatory action by Golgi tendon organ afferents.

[1] R. M. E. Carrea and H. Grundfest, J. Neurophysiol. *17*, 208 (1954).

The experiments were performed on decerebrate cats. Contralateral muscle nerves from the hindleg were stimulated with single shocks and the mass discharge in the v.s.c.t. was recorded by a surface electrode placed on the exposed superior cerebellar peduncle. The ingoing volley was judged by triphasic recording from the dorsal roots, and only cats with group I*a* and I*b* separation were used[2]. Such an experiment is illustrated in Figure 1.

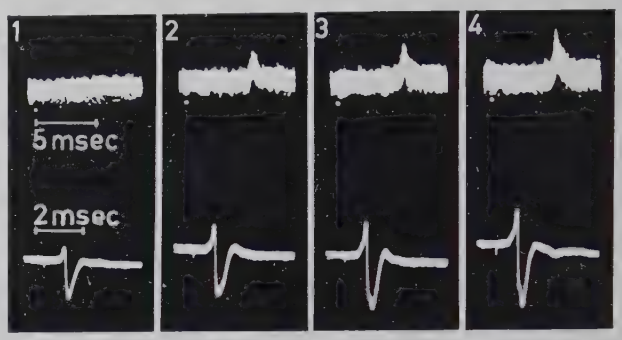


Fig. 1. – Activation of the v.s.c.t. with group Ib. Stimulation of the contralateral quadriceps nerve. Superposed records of simultaneous recordings from dorsal root (*LV*, lower records) and from the v.s.c.t. at the superior cerebellar peduncle.

With the maximally obtainable pure group I*a* volley, no mass discharge is to be seen (record 1). The addition of a small part of group I*b* already results in activation of v.s.c.t. neurons (record 2). The discharge is well synchronized and appears after 5 ms. It increases with activation of more afferents in group I*b*. The appearance of group II (record 4) represented by a small hump after group I in the dorsal root record, does not seem to have any influence on the discharge.

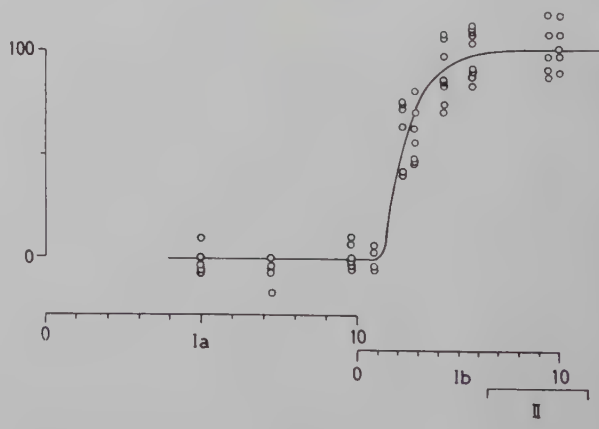


Fig. 2. – A curve showing the activation of the v.s.c.t. with group I*b*. Stimulation of the contralateral hamstring nerve. Several records were taken with each stimulus strength. For explanation see the text.

The potentials recorded in a similar experiment are plotted to give the curve of Figure 2. The ordinate represents the mass discharge in the v.s.c.t. and the abscissa the dorsal root volley with separate measurements for the magnitude of the group I*a* and I*b* potentials. The beginning of group II was assessed by separate recordings from a root filament at the end of the experiment.

[2] K. Bradley and J. C. Eccles, J. Physiol. *122*, 462 (1953). – J. C. Eccles, P. Fatt, S. Landgren, and G. J. Winsbury, J. Physiol. *125*, 590 (1954).

The last series of records were taken with supramaximal group II stimulation. The curve shows that the discharge appears early with group I*b* and rapidly increases to reach a maximum before group II is activated. As might be expected the discharge remained after a lesion of the spinal cord leaving the ipsilateral ventral quadrant, but disappeared after a superficial section in that quadrant. Simultaneous triphasic recording of the discharge in the v.s.c.t. from the ventro-lateral surface of the spinal cord and from the superior cerebellar peduncle made it possible to assess the propagation velocity of the fastest fibres in the tract to approximately 120 m/s. This is a slightly higher velocity than that found in the v.s.c.t.[3] The time delay between the dorsal root volley and the discharge in the v.s.c.t. when reduced by the propagation time is about 1 ms or less. This delay suggests one synaps only.

B. Holmqvist and O. Oscarsson

Institute of Physiology, Lund, Sweden, May 31, 1956.

Zusammenfassung

Nach kontralateraler Muskelnervreizung trat die Massenentladung der ventralen Seitenstrang-Kleinhirnbahn erst nach Auftreten der zweiten Komponente der Gruppe I-Welle auf. Dies spricht für eine Reizwirkung, die durch die afferenten Fasern der Golgi-Sehnenspindeln ausgelöst wird. Die Massenentladung verlief synchron und hatte eine Dauer von ungefähr 2 ms. Die Leitungsgeschwindigkeit der schnellsten Fasern der ventralen Seitenstrang-Kleinhirnbahn betrug etwa 120 m/s.

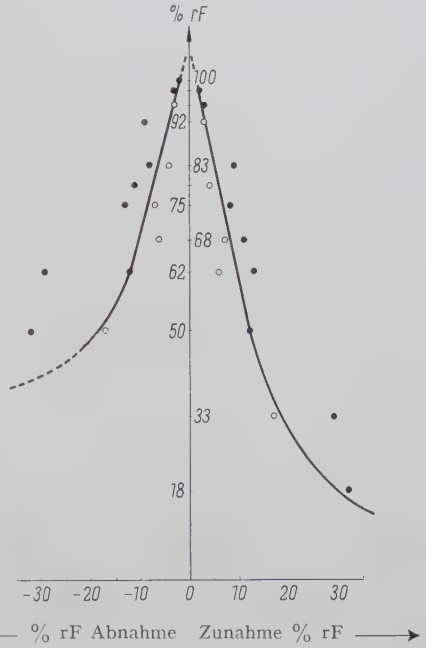
[3] D. P. C. Lloyd and A. K. McIntyre, J. Neurophysiol. *13*, 39 (1950). – Y. Laporte, A. Lundberg, and O. Oscarsson, Acta physiol. Scand. *36*, 175 (1956).

Der Trockenheitssinn bei Larven von *Drosophila Melanogaster*

Wird Larven von *Drosophila melanogaster* die Wahl zwischen zwei Kammern mit verschieden feuchter Luft gelassen, so wählen die Larven die feuchtere Seite. Um den die Feuchtigkeit wahrnehmenden Sinn genauer zu prüfen, wurde eine zweikammerige Arena gebaut. Sie bestand aus einer flachen, runden Glasschale mit gutschliessendem Deckel, deren Boden mit Wachs ausgegossen worden war. Diagonal wurde in die Schale eine Trennwand aus Glas so eingekittet, dass diese oben am Deckel dicht anschloss, während sie unten bis etwa 1,5 mm über den Boden reichte. Eine auf dem Boden kriechende Larve konnte so ohne weiteres von einer Kammer in die andere hinüberwechseln.

Die relative Feuchtigkeit (rF) in den beiden Kammern konnte mit Filterpapierstreifen, die mit destilliertem Wasser oder einer gesättigter Salzlösung getränkt und oben am Deckel angeklebt wurden, geregelt werden. Getestet wurde bei 25°C. Je nach der Art der verwendeten Salzlösungen erhält der sie umgebende Raum eine bestimmte rF (Buxton[1]).

Die Resultate der Untersuchungen sind in der Abbildung eingetragen. Jeder Punkt in der Darstellung entspricht 10 Testen mit je einer etwa 90 h alten Larve des 3. Stadiums. Die Differenz gilt als wahrgenommen, wenn mehr als die Hälfte der geprüften Tiere eine der unten beschriebenen Reaktionen zeigte.



Resultate von Testen über das Wahrnehmungsvermögen von Feuchtigkeitsunterschieden bei Larven von *Drosophila melanogaster*. Ordinate: relative Feuchtigkeit (rF) in der Ausgangskammer, in der sich das Tier zu Beginn des Versuches befindet. Abszisse: rF in der Wahlkammer, links weniger feucht, rechts grössere rF.
● Differenz wahrgenommen, ○ Differenz nicht wahrgenommen.

Aus den Resultaten ist ersichtlich, dass die Larven bei hoher rF noch Unterschiede von 2% wahrnehmen, während bei relativ trockener Luft nur noch relativ grosse Unterschiede wahrgenommen werden können. Die wahrgenommene Differenz gegen oben ist daher immer kleiner als diejenige nach unten. Nach dem Weberschen Gesetz können wir annehmen, dass die wahrnehmbaren Reizunterschiede um so grösser sein müssen, je stärker der Reiz bereits ist. Da bei Drosophilalarven die Reizschwellen bei geringer rF sehr gross sind, können wir annehmen, dass bei ihnen das Fehlen von Feuchtigkeit die Sinnesorgane reizt; es handelt sich also um einen Trokkenheitssinn.

Je nach der Grösse der Feuchtigkeitsunterschiede an der Scheidewand können wir zwei Reaktionsweisen unterscheiden. Ist die Differenz gering, so macht die Larve, sobald sie von der feuchteren Kammer in die trockenere hinüberwechselt, Suchbewegungen, die sie rasch wieder in die feuchtere Kammer zurückbringen. Ist die Differenz aber gross, zum Beispiel 98/50%, so kriecht die Larve gar nicht in die trockenere Kammer hinüber. Vielmehr hält sie an der Grenze an, hebt das Kopfsegment und die Thoraxsegmente senkrecht vom Boden ab, beginnt mit dem Vorderleib in der Luft hin- und herzuschwingen und kehrt dann brüsk in die feuchte Kammer zurück. Hafez[2] hat bei Larven von *Musca domestica* das gleiche festgestellt. Seine Untersuchungen ergaben, dass die sogenannten Fußstummel-Sinnesorgane von Keilin[3] auf der Ventralseite der Thoraxsegmente Feuchtigkeitsrezeptoren seien. Die gleichen Be-

[1] P. A. Buxton, Bull. entomol. Res. *22*, 431 (1931). – P. A. Buxton and K. Mellanby, Bull. entomol. Res. *25*, 171 (1934).

[2] M. Hafez, Parasitology *40*, 215 (1950).

[3] D. Keilin, Bull. sci. France et Belg. *49*, 15 (1915).

funde konnten nun auch für *Drosophila melanogaster* erhoben werden. Wenn die Fußstummel-Sinnesorgane mit Vaseline überdeckt werden, kann die Larve keine Feuchtigkeitsdifferenzen von weniger als 50% wahrnehmen. Hingegen nimmt die Larve noch sehr grosse Unterschiede wie 100% zu 33, bzw. 18% wahr. Wird das Hinterende von Drosophilalarven auch noch mit Vaseline überstrichen, so nimmt die Larve keine Feuchtigkeitsunterschiede mehr wahr. Für die Wahrnehmung von extremer Trockenheit, bei abgedeckten Fußstummel-Sinnesorganen scheinen die sogenannten Fleischzapfenorgane am Hinterende der Tiere (Heeger[4]) verantwortlich zu sein. Aus den oben angeführten Gründen möchten wir bei den Fußstummel-Sinnesorganen von Trokkenheitsrezeptoren sprechen.

G. Benz

Zoologisches vergleichend-anatomisches Institut der Universität Zürich, den 25. April 1956.

Summary

Larvae of *Drosophila melanogaster* perceive very small differences of relative humidity if the humidity is high, whereas the perceptible differences are relatively large in dry air. The sense organs for these perceptions are the so-called tufted organs on the ventral side of the thoracic segments. From the fact that the threshold of sensation is high in dry air, we conclude that the absence of humidity irritates the sense organs. We think, therefore, that they are aridity receptors.

[4] E. Heeger, Sitz.-Ber. Kais. Akad. Wiss. math.-nat. Kl. *31* (1858).

Advantages Given by P^{33}–or Aged P^{32}–in Autoradiography

In earlier experiments[1], we have seen that P^{33} gives a higher yield than P^{32} if exposed to nuclear emulsions whose thickness contains entirely the terminals of the beta tracks of P^{33} and not all those of P^{32}.

It appeared interesting to see whether P^{33} could present advantages in autoradiography, although the X-ray films are far from being thick enough to contain entirely the tracks of P^{33}. Experiments were therefore made to see whether, in the case of diffused incidence, samples of aged P^{32} containing 50% P^{33} could give a satisfactory response in comparison with the samples of freshly prepared P^{32}.

22 couples of specimens were prepared using material from Harwell. The solutions were diffused in discs of gelatine from Kodak Scientific plates fixed, about 15 μ thick, which could fairly simulate soft tissues; 18 of these couples were applied to a glass support and 4 at bottom of a very low aluminum dish.

Care was taken to avoid errors owing to different self-absorption conditions; therefore the much more active P^{32} solution was diluted with an inactive solution of sodium phosphate in order to use, for the samples to be compared in each experiment, not only equal activities in identical geometrical conditions, but also equal amounts of solution of equal concentration.

[1] Jane Mayr, Exper. *11*, 21 (1955).

The activities, which varied from 0·19 to 0·48 mμc were measured with a gas-flowing counter: the precision degree – depending upon the activity over background – was of 2–3%.

Fig. 1.

Each couple of tests was then exposed for the same time to a "Ilford X-ray" film, 11 μ thick (16 couples to a simple coated and 6 to a double coated).

After development, a first rough comparison of the dark images was made by means of a densitometer, considering only the couples showing sufficient uniform dark images: in these cases about 30–50 regions of each image were examined. They showed approximately the same values of "*D*" (with a deviation not exceeding 8%). The behaviour of double coated films was like that of the simple coated ones.

In order to give precise data and a visible demonstration, we have chosen a couple of film dark regions (corresponding respectively to tests of P^{32} and of mixture P^{32}–P^{33}), have determined with a microdensitometer the densities along lines 1 mm distant from each other, and have calibrated the instrumental data with an "Ilford wedge" so that precise values of density could be known. On the basis of these data, we have been able to draw isodensity curves on discs having a diameter 10 times greater (Fig. 1), to prepare the plastics considering that each value of $D=0{\cdot}05$ should be represented by the thickness of a card board foil (Fig. 2) and to weigh the two plastics.

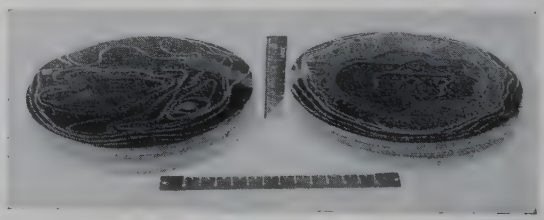


Fig. 2.

The weight of these two plastics was 411 g (for the one corresponding to the mixture test) and 420 g (for the one corresponding to the P^{32} test); thus, since the background is in each case 300 g, it appears that the difference between the two samples was about 8%.

Furthermore, a grain count was made using a microscope with a magnification of 100 × 8·5 and considering for each image 50–70 reticle fields of 144 μ^2. The grain yield was respectively for the mixture and for the P^{32} of $1{\cdot}1 \pm 0{\cdot}3$ and of $1{\cdot}2 \pm 0{\cdot}3$ grains developed per incident electron; probably backscattering and cross fire diminish the difference between the two samples.

At any rate it can be stated that the aged phosphorus containing 50% P^{33} gives quite a satisfactory response in comparison with P^{32} alone, and that, for autoradiography – as long as the experiments refer to the usual radiographic procedure in which the specimen is transparent and presents very little self-absorption – P^{33} (or also aged P^{32}) is preferable to pure P^{32} because the "reps" delivered are considerably less that for P^{32}.

This conclusion may be useful in all those types of investigation in which the dosage rate from the same activity of P^{32} would produce radiation damage[2].

I wish to thank Prof. J. S. Mitchell for having suggested the consideration of P^{33}, for his valuable advice and for having given me hospitality in the Department of Radiotherapeutics of the University of Cambridge, where this research was carried on.

Jane Mayr

Department of Radiotherapeutics, University of Cambridge, March 26, 1956.

Riassunto

Si è sperimentalmente dimostrato che il vecchio radiofosforo contenente il 50% di P^{33} è preferibile al comune radiofosforo P^{32} perchè, a pari attività, presenta effetto fotografico pressocchè eguale e dosaggio meno dannoso.

[2] L. F. Lamerton and E. Harris, J. photogr. Sci. *2*, 135 (1954).

Changes in Muscle Circulation Elicited by Local Heating of Hypothalamus

Barcroft *et al.*[1] recently demonstrated vaso-motor changes in the muscle of man from changes in environmental temperature. A decrease in muscle blood-flow of about 55% was obtained by warming the subjects. The results of Rein and Schneider[2], formerly criticized by many authors[1], have thus been confirmed. No evidence is, however, given as to whether the effects are elicited from skin receptors, from hypothalamic thermosensitive structures, from baroceptive influences compensating the vaso-motor reactions in the skin, or from changes in skeletal muscle tone, which is well known to be important for heat production. Recently von Euler and Söderberg[3] found that local heating of hypothalamus caused a marked decrease in gamma motor activity (tested on muscle spindle afferents by the usual method[4] even without measurable changes in muscle tension. The aim of the present investigation therefore was to see whether hypothalamic heating would influence muscle blood-flow without any changes in muscular tone. The importance of muscle tension was also studied since it is reasonable to assume from Denny-Brown's work[5], that vaso-motor effects may be reflexly elicited by stretch.

In nembutalized cats, local heating of hypothalamus was produced by high frequency alternating current (1 megacycle per s). At the tip of one of the heating electrodes, a thin enamelled advance wire was soldered to form one part of a thermocouple by which the maximal temperature induced by the current could be recorded[2].

The femur was immobilized, the gastrocnemius muscle dissected free from surrounding tissues and covered with warm paraffin. The Achilles tendon was cut and attached to a sensitive strain gauge. Heparin was given and the popliteal vein cannulated. Ligations were made so that the blood from the canula came from the gastrocnemius muscle only. The blood drops from the free end of the canula were counted electrically, the

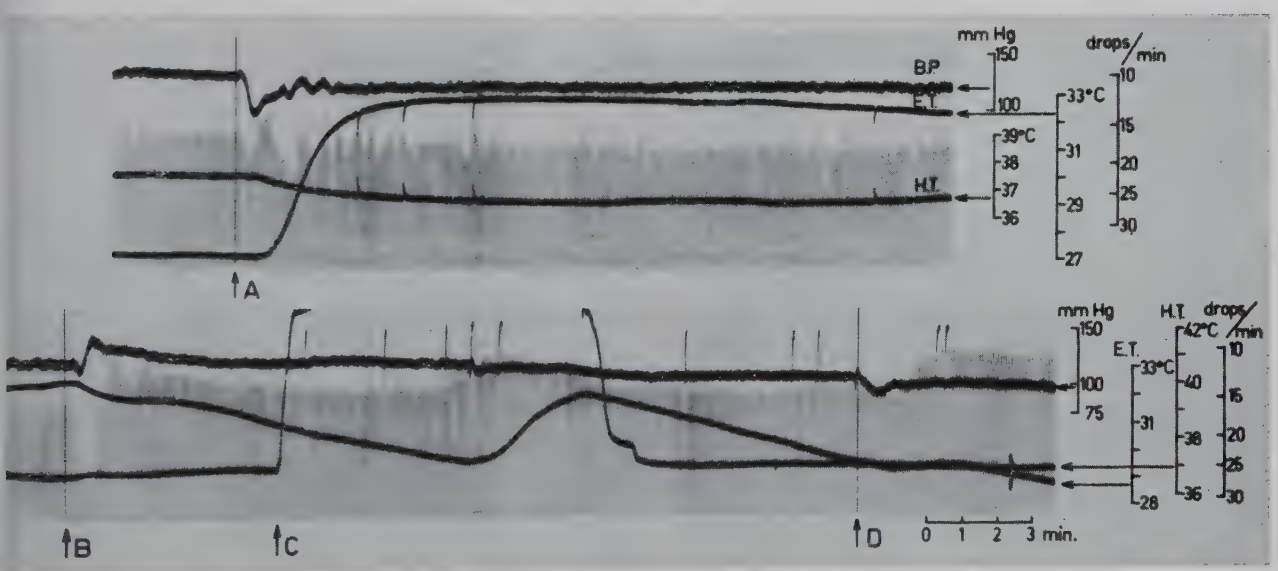
[1] H. Barcroft, K. D. Bock, H. Hensel, and A. H. Kitchin, Pflüger's Arch. ges. Physiol. *261*, 199 (1955).

[2] H. Rein and D. Schneider, Z. Biol. *91*, 13 (1930).

[3] C. von Euler and U. Söderberg, Exper. *12*, 278 (1956).

[4] R. Granit and B. R. Kaada, Acta physiol. scand. *27*, 130 (1952).

[5] D. Denny-Brown, Proc. roy. Soc. [B] *104*, 252 (1929).



Cat, 2·5 kg. "Nembutal" (40 mg/kg). *BP* Blood pressure from femoral artery. *ET* Ear skin temperature (thermo-couple on right ear). *HT* Hypothalamic temperature as described in the text. *Vertical lines*: Blood-flow from the gastrocnemius muscle. Each drop of blood from the muscle vein rapidly carries spot of mirror galvanometer to base of Figure, from which it returns at slower speed so that a steeply rising line is recorded. The length of these lines is directly proportional to time-interval between drops. By joining the upper end of the lines, one obtains inverted tracings of blood-flow, *cf.* calibration on the right. *A* 25 mg "Nembutal" intravenously. *B* 5 μg adrenaline intravenously. *C* Hypothalamic heating. *D* 2·5 mg chlorpromazine intravenously. Note slight decrease in muscle flow 2 min after the beginning of hypothalamic heating, coincident with vasodilatation in the skin (increased ear skin temperature).

time interval between them being registered by an ordinate recorder. The blood was readministered by intravenous drop infusion. Arterial blood pressure, rectal temperature and ear skin temperature (as an index of skin circulation) were recorded.

A typical result is demonstrated in the Figure *C*, where hypothalamic heating gave decreased flow through the muscle and increased skin temperature (secondarily to vasodilatation) with a latency of about 2 min. Blood pressure was slightly lowered. At the end of the heating, the muscle blood-flow returned to the earlier level although the blood pressure was about 20 mm Hg lower. The muscle tension record (not reproduced in the Figure) was constantly at 10 g throughout the period of observation shown in the Figure.

The effects of hypothalamic heating on muscle circulation were as a rule small as compared to those seen in the skin. The effects were probably not due to baroceptive influences since the changes in skin and muscle blood-flow were not always related in magnitude. Vasomotor effects in the muscle could only be demonstrated when it was under some stretch. This may explain why Folkow *et al.*[6] did not find any changes in muscle flow during hypothalamic heating, although electrical stimulation reveals the presence of nervous structures capable of mediating such effects[7]. Small additional doses of "Nembutal" which gave a rapid vasodilatation in the skin generally induced a slight increase in blood-flow also in the muscle (*A*), whereas small doses of chlorpromazine sometimes elicited vasoconstriction (*D*). As a rule, however, and regularly in large doses, chlorpromazine also induced vasodilatation.

U. Söderberg

Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm 60, Sweden, April 25, 1956.

Zusammenfassung

Es wurde gefunden, dass lokale Erwärmung des vorderen Teils des Hypothalamus an mit „Nembutal" narkotisierten Katzen die Durchblutung des Musculus gastrocnemius verminderte, ohne dass messbare Änderungen des Muskeltonus auftraten. Der Effekt erfolgte nur, wenn der Muskel unter einer gewissen Spannung war. Kleine Dosen von Chlorpromazin hatten manchmal ähnliche Wirkung.

[6] B. Folkow, G. Ström, and B. Uvnäs, Acta physiol. scand. *17*, 317 (1949).

[7] P. Lindgren, Acta physiol. scand. *35*, Suppl. 121 (1955).

Effects of Pyrithiamine and Oxythiamine on the Thiamine Content of Tissues and Blood Pyruvate in Mice

Woolley and Merrifield, in two consecutive papers[1], postulate a new metabolic function of thiamine not mediated through the cocarboxylase. The experimental evidence which supports this statement is represented by the different *in vivo* actions of two antivitamins, pyrithiamine (PY) and oxythiamine (OT). While both compounds produce a thiamine deficiency in animals, only the PY calls forth the typical neurological disturbances of the athiaminosis in mice, without however modifying the cocarboxylase content of the liver[2] or the blood pyruvate[3]. On the contrary, according to Frohman and Day[4], OT increases both the urinary excretion of thiamine and the blood pyruvate in the rat, but fails to produce the neuromuscular syndrome.

These results led Woolley and Merrifield[5] to the conclusion that PY "in calling forth characteristic manifestations of thiamine deficiency interferes primarily with a function of thiamine responsible for the typical neurological signs and that this function is distant from the one involving cocarboxylase and pyruvate".

In other words, because the PY, administered to mice, does not modify the liver thiamine content and the blood pyruvate, which is notoriously connected with the cocarboxylase activity and produces the typical disturbances of the thiamine deficiency, the authors conclude that the PY interferes with a function of the thiamine indispensable to the nervous tissues and not involving the carboxylase. Instead, the OT would be mainly the antagonist of cocarboxylase.

In order to verify this interesting hypothesis, we repeated and extended Woolley's[2] experiment, determining the thiamine contents not only of the liver but also of the muscle and particularly of the brain, since the new function of the thiamine concerns the nervous tissue.

Thus male mice of 11–14 g body weight were housed in single small cages and reared on thiamine deficient diet of the following composition: Washed, fat-free casein 18%; wheat starch 65%; olive oil 10%; cod liver oil 2%; Osborne and Mendel salt mixture 5%, supplemented with suitable amounts of all the B vitamins and α-tocopherol. In experiment No. 1, 0·5 mg of PY[6], dissolved in 0·02 ml of H_2O, was administered *per os* on the first day, followed by a daily dose of 2 μg of thiamine *per os*: the controls received only 2 μg of vitamin *pro die*.

In experiments No. 2, 3 and 4, the administration of thiamine was discontinued and the animals fed the thiamine deficient diet were treated at the beginning of the experiment with a single dose of PY or OT[7] *per os*, dissolved in 0·02 ml of H_2O, as indicated in the Table. The controls were placed on the same diet. The mice were killed by decapitation, together with their controls, when the neuromuscular symptomatology appeared. In the experiments in which the disturbances did not appear the mice were killed on the 10th day of the experiment.

The blood pyruvate was determined by the micromethod of Rindi and Ferrari[8]: the thiamine (total) contents of the tissues (liver, muscle and brain) were determined by a modification of the classical thiochrome method[9], where the isobutanol extraction was performed with 5 ml of solvent. The method used has been checked on pure thiamine solutions and always gave good results, closely comparable to those obtained with the usual method.

[1] D. W. Woolley and R. B. Merrifield, Fed. Proc. *11*, 458 (1952); Bull. Soc. Chim. biol. *36*, 1201 (1954).

[2] D. W. Woolley, J. biol. Chem. *191*, 43 (1951).

[3] D. W. Woolley and R. B. Merrifield, Bull. Soc. Chim. biol. *36*, 1201 (1954).

[4] C. E. Frohman and H. G. Day, J. biol. Chem. *180*, 93 (1949).

[5] D. W. Woolley and R. B. Merrifield, Fed. Proc. *11*, 458 (1952).

[6] Neopyrithiamine, made by the California Foundation for Biochemical Research, Los Angeles, Cal.

[7] Kindly supplied by Roche Products Ltd., Welwyn Garden City, England.

[8] G. Rindi and G. Ferrari, Exper. (in press).

[9] Association of Vitamin Chemists, Inc., *Methods of Vitamin Assay* (2nd Ed., Interscience Publ., New York 1951), p. 111.

Body weight, blood pyruvate and total thiamine content of the tissues of PY or OT treated mice. Mean values ± Standard error

Experiment No.	Treatment	No. of mice	Body weight, g		Blood pyruvate mg/100 ml	Thiamine (total) content, μg/g			% of mice showing neurological symptoms
			initial	final		liver	muscle	brain	
1	0·5 mg PY + 2 μg B_1	9	12·0 ± 0·60	15·8 ± 0·72	2·10 ± 0·15	3·39 ± 0·27	0·96 ± 0·04	1·58 ± 0·07	0
			0·7 > p > 0·6		0·4 > p > 0·3	0·9 > p > 0·8	0·02 > p > 0·01	0·001 > p	
	Controls (2 μg B_1)	9	11·7 ± 0·32	15·8 ± 0·49	1·92 ± 0·08	3·30 ± 0·25	1·15 ± 0·06	2·46 ± 0·11	0
2	0·5 mg PY	9	13·9 ± 0·22	12·2 ± 0·24	3·08 ± 0·50	2·11 ± 0·13	0·78 ± 0·03	0·74 ± 0·05	100
			0·9 > p > 0·8	0·001 > p	0·1 > p > 0·05	0·1 > p > 0·05	0·9 > p > 0·8	0·001 > p	
	Controls	9	14·1 ± 0·44	14·2 ± 0·40	2·02 ± 0·19	1·69 ± 0·17	0·79 ± 0·06	2·16 ± 0·08	0
3	0·5 mg OT	9	13·0 ± 0·34	14·0 ± 0·45	2·19 ± 0·37	1·34 ± 0·05	0·94 ± 0·06	2·34 ± 0·12	0
			0·6 > p > 0·5	0·5 > p > 0·4	0·8 > p > 0·7	0·01 > p > 0·001	0·5 > p > 0·4	0·9 > p > 0·8	
	Controls	9	12·7 ± 0·36	13·6 ± 0·34	2·07 ± 0·14	1·73 ± 0·09	1·00 ± 0·04	2·35 ± 0·15	0
4	2 mg OT	9	12·1 ± 0·23	12·5 ± 0·19	2·26 ± 0·10	1·23 ± 0·06	0·85 ± 0·02	2·13 ± 0·07	0
			0·4 > p > 0·3	0·6 > p > 0·5	0·5 > p > 0·4	0·001 > p	0·5 > p > 0·4	0·6 > p > 0·5	
	Controls	9	12·5 ± 0·34	12·8 ± 0·47	2·14 ± 0·10	1·67 ± 0·11	0·89 ± 0·05	2·20 ± 0·08	0

The significance of the difference between the means has been determined according to the t of "Student"

As can be seen from the Table, in experiment No. 1, which is the same as that performed by WOOLLEY[2], the mice did not show, during the 10 days of observation, any neuromuscular disturbance; the blood pyruvate and the thiamine content of the liver were not different from the controls. These results closely agree with WOOLLEY's findings[2]. However, in muscle and brain the thiamine content is sharply lowered: the decrease is statistically significant.

In experiment No. 2, where we did not administer the thiamine and used the same dose of PY as in experiment No. 1, all the treated mice showed the neuromuscular syndrome after 6 to 10 days of the antivitamin treatment. Their body weight was lowered, the blood pyruvate increased although not significantly, in comparison with the controls which did not show any neurological disturbance. It is well known, in fact, that in the alimentary athiaminosis of mice the motorial syndrome does never appear.

The thiamine content was unchanged in the liver and in the muscle, whereas it was greatly decreased in the brain. This means that the PY, in our experimental conditions, did not induce a further decrease of the thiamine contents of liver and muscle, already lowered by the deficient diet, but it was very efficacious in reducing to a further extent the brain thiamine level. Actually, the diminution in experiment No. 2 is much greater than in experiment No. 1 where it is likely that the daily administration of thiamine tends to abolish the effects caused on the brain vitamin content by the single dose of PY.

In experiments No. 3 and 4, the mice, treated with two different doses of OT, never showed any neuromuscular syndrome and behaved like the controls. Only in the liver we found a slight but statistically significant decrease of the thiamine content, bearing out in the mice what we had already observed in the rat[10].

The results obtained under our experimental conditions show that PY lowers the thiamine content particularly in the nervous tissue (brain) with a mechanism which is still unknown. The neuromuscular disturbances and the body weight decrease were found only in those animals in which the decrease of the brain thiamine content was conspicuous.

The OT, administered under the same experimental conditions, fails to produce, even in doses 4 times greater than PY, the neuromuscular syndrome or any modifications of the blood pyruvate, of the body weight or of the thiamine contents of both brain and muscle, although it slightly lowers the liver content. Therefore our findings do not support the hypothesis of a new function of thiamine in the nervous tissue. Actually the PY, in the dose used, fails to lower the thiamine level of the liver, according to WOOLLEY's results, but it greatly lowers the brain content, producing the neurological disturbances typical of thiamine deficiency.

On the other hand, the OT shows an action only on the liver, where it slightly lowers the thiamine content without affecting the blood pyruvate.

L. DE CARO, G. RINDI, V. PERRI, and G. FERRARI

Institute of Human Physiology, University of Pavia (Italy), April 27, 1956.

[10] L. DE CARO, G. RINDI, V. PERRI, and G. FERRARI, Int. Z. Vitaminforsch. (in press).

Riassunto

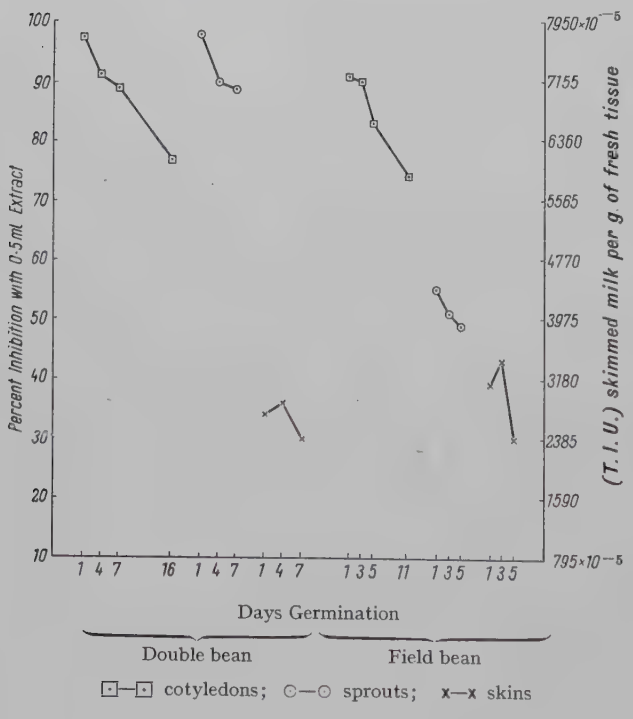
La Piritiamina, somministrata per bocca in una singola dose di 0,5 mg a topolini di 12–14 g che ricevono 2 μg di tiamina *pro die*, non produce la sindrome neuromuscolare caratteristica dell'avitaminosi B_1 e non modifica nè la piruvicemia nè il livello di vitamina nel fegato, mentre lo fa abbassare nel muscolo e nel cervello. Se i topolini non ricevono tiamina, la stessa dose di Piritiamina dà perdita di peso, sindrome neuromuscolare in tutti gli animali trattati e cospicuo abbassamento del livello di vitamina B_1 nel cervello. Nelle stesse condizioni sperimentali, l'Ossitiamina, somministrata *per os* una sola volta in dosi di 0,5 e 2 mg, non dà alcuna sintomatologia neuromuscolare e non modifica nè il peso corporeo, nè il piruvato ematico, nè il contenuto in vitamina B_1 del muscolo e del cervello, solo abbassa il livello vitaminico del fegato.

Questi risultati non sono in favore dell'ipotesi di una funzione della tiamina distinta da quella della cocarbossilasi.

Trypsin Inhibitor in Plant Metabolism

Sohonie and Ambe[1] have recently reported the crystallization of trypsin inhibitors from the Indian double bean (*Faba vulgaris*) and field bean (*Dolichos lablab*). The same pulses have been further investigated for the inhibitor activity throughout their plant life-cycle. For comparison, the inhibitor activity residing in equal amounts of cotyledons, sprouts and skins of the germinating grains and also of roots, stems, leaves, legumes, etc., of the growing plants has been determined at three stages of development.

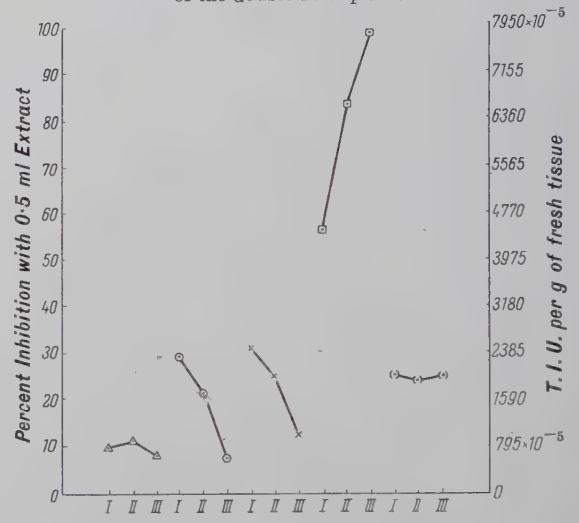
Fig. 1.–Trypsin inhibitor activity of various parts of the germinating double bean and field bean.



[1] K. Sohonie and K. S. Ambe, Nature *175*, 508 (1955).

The various plant organs were extracted under identical conditions with dilute hydrochloric acid and the inhibition caused due to equal portions of extracts were evaluated. A method similar to that described by Anson[2] was employed using digestion mixtures of skimmed milk and commercial trypsin (Merck's) at pH 7·6, at 37° C. The inhibitor activities of the various parts of the germinating pulses and their plants have been presented graphically (Fig. 1, 2. 3).

Fig. 2.–Trypsin inhibitor activity of various organs of the double bean plant.



Plant Organs at Three Stages of Development
(I = tender; II = semimature; III = mature)

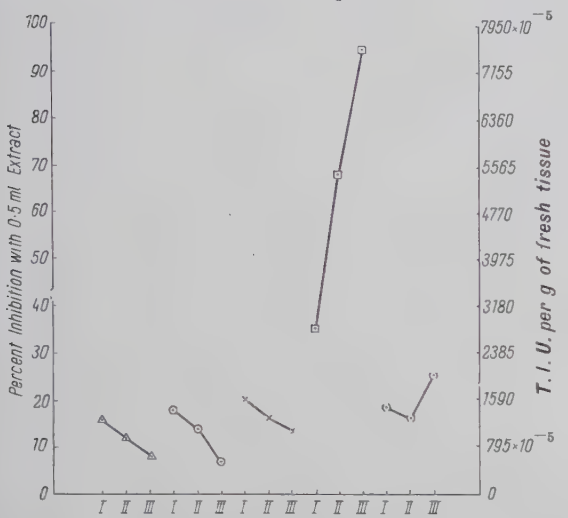
It is interesting to note that the trypsin inhibitor is present in all the parts of the germinating pulses and their plants at all the stages of growth. A study of the curves depicted here reveals that the inhibitor activity in the different parts of either plant is found to be maximum in the seeds and least in the roots; the leaves, stems, etc., fall in between. The cotyledons and the sprouts show decrease in the inhibitor content with germination, while the skins do not exhibit any significant variations. On the plants, in the same organ, however, with the exception of the legume seeds, the power of inhibition diminishes with maturation. The seeds, on the other hand, show an increase in the inhibiting capacity with development. A parallel investigation on the plants of potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), green grams (*Phaseolus aureus*) and Asiatic Yam (*Dioscorea alata*) at different stages of development has also confirmed the same trend of results. The activity of the extracts of leaf-stem portions is found to decrease with maturation whereas the tubers and the seeds exhibit a reverse phenomenon (unpublished).

These results suggest a parallelism between the changes occurring in the inhibitor activity and the protein synthesis in the plant tissues. Burström[3] has shown that the latter declines with age; and the same seems to be true, with the exception of the seeds, in the case of the trypsin inhibitor concentrations in different parts of the plants analyzed. The seeds form the storage

[2] M. L. Anson, J. gen. Physiol. *22*, 79 (1938).
[3] H. Burström, Bot. Arch. [B] *30*, 1 (1943).

organs of the plants and the accumulation of the proteins explains the increase in the inhibitor activity of the seeds as they grow.

Fig. 3.–Trypsin inhibitor activity of various organs of the field bean plant.



△—△ roots; ○—○ stems; x—x leaves; ⊡—⊡ seeds; (·)—(·) skins

Plant Organs at Three Stages of Development
(I = tender; II = semimature; III = mature)

Whether the inhibitors in the plant tissues function as stabilizers of the associated proteins and act as regulators in the protein synthesis and breakdown or otherwise, is a question that should be left open at this stage. In fact, a study of the interaction of the plant inhibitors with the plant proteases themselves should preceed such an enquiry.

A detailed account of the investigation will be published elsewhere.

The authors wish to express their gratitude to the Council of Scientific and Industrial Research (India), for financing this work and a personal grant to one of us (K.S.A.).

K. S. Ambe and Kamala Sohonie

Institute of Science, Department of Biochemistry, Bombay, April 3, 1956.

Résumé

L'activité inhibitrice de la trypsine contenue dans les différentes parties de *Faba vulgaris* Mœnch et *Dolichos lablab* L., pendant germination, et des plantules et de leurs parties en voie de croissance a été déterminée à trois différents stades, à savoir aux états: 1° de tendre, 2° de demi-maturité et 3° de maturité. On l'a constatée aux trois stades. Sauf dans les graines, l'activité inhibitrice diminue avec la maturation. Dans les graines c'est l'inverse, l'activité inhibitrice de la trypsine augmente avec la maturité. On suggère le rôle possible de ce principe comme régulateur dans le processus de formation et de rupture de la protéine.

Paper Electrophoresis of the Soluble Proteins of the Central Nervous Tissue

Reports in the literature on the electrophoretic separation of the proteins of the nervous tissue are, as far as we are aware, very scarce. Keil[1] described the separation, by paper electrophoresis, of the proteins extracted from the obturatorious nerve of ox and demonstrated 4 components.

In the same year Nakamura, Hayashi, and Tanaka[2] studied, by boundary electrophoresis, the soluble proteins from ox brain, demonstrating 8 fractions.

Paper electrophoresis of the soluble proteins of the cerebellum after 12 h of electrophoresis. Borate buffer pH 8·6 μ 0·06.

In the present communication the techniques used and the results obtained in the separation, by paper electrophoresis, of the soluble proteins of central nervous tissue of albino rats are outlined. Brain and cerebellum (pooled separately) of albino rats weighing about 200 g were used. Animals were killed by exsanguination under ether anaesthesia and the head was immediately perfused through the ascending aorta with 100 ml of ice-cold 0·25 *M* sucrose solution. Brain and cerebellum were quickly dissected, frozen over solid CO_2 and set aside to await completion of collection.

The pooled material was then dispersed in a Potter-Elvehjem glass homogenizer kept at ice-water temperature, with 0·25 *M* sucrose (5 ml/g of tissue). The dispersion was centrifuged for 1 h at 25000 × *g* in a refrigerated high-speed centrifuge (Pirouette Phywe) and the decanted supernatant, which had a slightly opalescent appearance, was dialyzed for 12 h in a cold room at 2° C, with mechanical agitation, against several changes of distilled water. After the dialysis the liquid was again centrifuged for 1 h at 25000 × *g* and frozen-dried.

Before use, the dry material was dissolved in boric acid–Na_2SO_4–NaOH buffer (Adjutantis: personal communication) pH 8·6, μ 0·06, to give a final protein concentration of 10% (*w*/*v*).

[1] A. W. Keil, Pflüger's Arch. *259*, 146 (1954).

[2] S. Nakamura, Y. Hayashi, and K. Tanaka, J. Biochem. *41*, 13 (1954).

If homogenization was carried out with a minimum of isotonic sucrose, protein concentrations adequate for electrophoresis could be obtained, omitting dialysis and freezing-drying; in this case the results were similar to those observed with the usual procedure.

Electrophoresis was carried out with a constant potential of about 6–8 V/cm for periods variable from 4 to 12 h. After drying in an oven at 120° C, the strips were stained with amido-schwarz 10 B according to Grassman, Hannig, and Knedel[3]. The relative amounts of the components were determined cutting the corresponding portions of the stained paper, eluting the dye with 5 ml of 5% solution of phenol in water and reading the optical density at 640 mμ.

The Figure shows the patterns of the soluble cerebellum proteins after 12 h of migration in borate buffer. 7 components are indicated with the numbers from 1 to 7 in the order of their increasing mobility. Some material is detectable ahead of fraction No. 7 indicating the presence of proteins with higher mobility, which represent 16·2% (± 1·1) of the total amount.

After runs lasting only 6–8 h, this material appeared distributed in 2 fairly definite bands. The band visible at the point of application of the protein solution on the paper is considered to represent particulate matter not sedimented by the centrifugal force employed.

The Table shows the relative amounts of the separated fractions obtained by dye elution.

Relative amounts of electrophoretic components of cerebellum proteins, obtained by dye elution, after 12 h of electrophoresis (means ± S.E.M.)*.

Fraction No	% Content	S.E.M.
1	6·61	0·71
2	8·33	0·81
3	11·27	0·59
4	7·33	1·45
5	16·45	0·37
6	17·97	1·04
7	15·13	1·43
Fast moving material	16·20	1·10

* The immobile material located at the point of application of the protein solution on the paper corresponds to 5% of the total amount of the soluble proteins.

Similar results were obtained with proteins extracted from the brain.

As resulting from the researches of Nakamura *et al.*[2] and from the present results, the patterns of the soluble proteins of the central nervous tissue appear differently from those of the nerve[1] and from patterns observed in other organs (liver[4]; muscle[5]).

R. Caravaglios and P. Chiaverini

Istituto di Patologia Generale and Istituto di Semeiotica Medica, Università di Pisa, November 10, 1955.

[3] W. Grassman, K. Hannig, and M. Knedel, Dtsch. med. Wschr. *76*, 333 (1951).

[4] G. Adjutantis, Nature *173*, 539 (1954); *174*, 1504 (1954).

[5] G. Toschi and A. Mariani, R. C. Accad. Lincei, Cl. Sci. fis. mat. e nat. [8] *16*, 365 (1954).

Riassunto

Le proteine idrosolubili estratte dal sistema nervoso di ratti sono state separate mediante elettroforesi su carta.

Il quadro elettroforetico appare costantemente riproducibile nelle condizioni sperimentali impiegate.

The Transformation of a Tail into Limb After Xenoplastic Transplantation

(1) One unexpected result obtained from the graft of a tail bud of a Urodele (*Triton cristatus; Axolotl*) on to an Anuran embryo at the neurula stage (*Discoglossus pictus*) is that the tail which differentiates from the transplant, may become transformed into a limb[1]. This, however, only occurs if the graft is made into a limb field of the host, and starts at, or close to, the time at which the metamorphosis of the host is initiated. This transformation occurred in 15 of 180 *Triton* grafts, and in 2 of 120 *Axolotl* transplants.

1 *2* *3*

Figs. 1, 2 and 3.–Axolotl tail bud grafted on to Discoglossus, respectively 13, 28 and 38 days after transplantation.

The stages of the transformation can be set out as follows:

(a) The graft develops into a normal tail, complete with fin, nerve cord, notochord and symmetrically arranged myotomes. It is slender and lanceolate in form (Fig. 1). Chromatophores, arranged in the pattern typical of the donor, are to be observed. The tail moves both spontaneously and upon stimulation.

(b) When the initial process of morphological and histological differentiation is complete, the tail undergoes regression, a process which particularly affects the fin. The tail ceases to be lanceolate, and instead assumes a cylindrical shape (Fig. 2). Concurrently it becomes fleshy and turgid and develops an intensely black pigmentation. The peduncle bends at right angles and at the distal extremity appears a variable number of digits (Fig. 3). It is, however, neither sensitive nor motile.

The limb persists for a considerable time. Meanwhile at its base a normal limb grows from the limb field of the host. In some cases an additional limb develops, which may fuse with the transplant, thus producing a chimera. A study of the histology of the transformed tail shows the nerve cord to be degenerating; in most specimens, remains of the notochord are present, but the musculature is almost completely lacking, a few sporadically

[1] N. Farinella-Ferruzza, Boll. Zool. *17*, 113 (1950); Riv. Biol. *45*, 523 (1953).

distributed striated muscle fibres alone remaining. The bulk of the transplant is composed of mesenchyme probably derived from the de-differentiation of the tail tissues. Among them are also to be found many pigmented cells. Where externally the appearance of the transplant most resembles a limb, internally nodular or elongated masses of healthy cartilage cells are found in the process of organization to form skeletal structures. In those specimens in which such organization has reached a maximum, it is possible to distinguish the appropriate parts of the pectoral girdle, stilo- and zeugopodia, carpus and digital cartilages. Surrounding the existing parts of limb skeleton are bundles of muscle fibres, almost certainly the developing limb musculature. More often, however, the girdle is absent, the skeletal components are reduced in size and number, and the musculature may be lacking.

(2) What can be said of the mechanism by which these changes are effected? As has been said previously, the progressive transformation outlined above can only occur if the graft is made into the limb field of the host. From its first appearance the limb field possesses capacity for induction, as has been shown by the present work. Probably chemical substances elaborated by the field pass into the graft. In most specimens no mesodermal cells from the host were to be found in the transplant; in the remainder they were present in extremely small numbers. Under the influence of these substances the first stages of degeneration are perhaps initiated in the donor tail, and subsequently the processes of reorganization which result in the formation of the limb from the tail. In corroboration, it should be noted that when the graft is made into a region other than the limb field, tail degeneration does not occur. Instead the tail maintains its shape and internal structure well beyond the period of metamorphosis of the Anuran host[1].

From what material is the limb formed? A study of histology directs attention to the differentiated mesenchymal cells among which are some that would naturally form the vertebral cartilages. It is probable that the limb skeleton differentiates from such cells under the inductive influence of the limb field. The caudal musculature probably redifferentiates to form the limb musculature.

(3) Recently Holtfreter[2] has obtained similar results to those outlined above. He has observed that tails obtained by induction (from transplants of pieces of mouse kidney into the blastocoel of *Triton alpestris*) and which find themselves in the host limb field, are transformed into limbs. He did not obtain the same results from direct transplants of the tail buds. It is possible that the explanation of the latter result is that the direct transplants were homoplastic. Xenoplastic grafts perhaps increase the inductive capacities of the limb field, as is shown by the frequent appearance of supplementary limbs.

N. Farinella-Ferruzza

Zoological Institute, University of Palermo, Italy, April 30, 1956.

Riassunto

Bottoni caudali di Triton e di Axolotl trapiantati sul campo dell'arto di Discoglossus (allo stadio di neurula) si differenziano in code, le quali talvolta, all'epoca della metamorfosi dell'ospite, si trasformano in arti. Viene discusso il meccanismo di tale trasformazione.

[2] J. Holtfreter, J. exper. Zool. *129*, 623 (1955).

On the Interpretation of the Low-Angle Scatter of X-Rays from Bone Tissue

Engström and Finean[1] demonstrated that, in addition to the wide-angle X-ray diffraction pattern, bone tissues also give a diffuse low-angle scatter. The same authors[2] assumed that the low-angle scatter could be treated as a particle scatter pertaining to the inorganic or mineral fraction. In this way they concluded that the particles are rod-shaped, the long axis of the rods being aligned in the direction of the longitudinal axis of the bone, and parallel to the collagen fibres. In the intact human bone these particles appear to have a diameter of about 73 Å, and a length of about 210 Å.

Recently Robinson and Watson[3] have criticized the conclusions of Engström and Finean, because observations with the electron microscope do not support the view that the inorganic particles are rod-shaped.

The findings which one of us published in this journal (see Ascenzi and Chiozzotto)[4] on the electron microscopy of the organic bone substance, using the pseudo-replica technique, induced us to repeat and possibly to improve on the investigations made by Engström and Finean in order to find a more adequate interpretation of the low-angle diffraction pattern.

Material and method.— Longitudinal and cross sections (0·2 mm thick) of the femoral diaphysis of cattle were prepared by grinding. The low-angle scatter was recorded from the untreated sections as well as from similar sections from which the ossein had been removed according to Gabriel's method (boiling in glycerol with 6% KOH). This procedure is unable to produce any change in the crystalline structure of the inorganic bone fraction[5] or increase in the size of the crystallites[6].

A low-angle scatter apparatus, somewhat similar to that employed by Finean[7] was used. The scatter was recorded using Ni filtered CuKα radiation ($\lambda = 1{\cdot}54$ Å)[8]. The maximal recorded scattering space corresponded to 250 Å. The intensity variation of the low-angle scatter was measured using a Leeds and Northrup automatic recording microphotometer.

Results and discussion.— The low-angle scatter of X-rays from longitudinal sections of bone shows a marked asymmetry (Fig. *a*), indicating an elongation of the elements or units responsible for the scatter along the longitudinal axis of the bone. On the contrary, the low-angle scatter obtained from cross-sections of bone (Fig. *b*) reveals no appreciable orientation.

This behaviour suggests, in agreement with Engström and Finean, that the scattering elements are well aligned and symmetrical around their long axes. Therefore the same elements appear as if they were like ellipsoids of revolution, the long axes being

[1] A. Engström and J. B. Finean, Nature *171*, 564 (1953).

[2] J. B. Finean and A. Engström, Biochem. biophys. Acta *11*, 178 (1953); Exper. *10*, 63 (1954). – R. Carlström and J. B. Finean, Biochim. biophys. Acta *13*, 183 (1954).

[3] R. A. Robinson and M. L. Watson, Ann. New York Acad. Sci. *60*, 596 (1955).

[4] A. Ascenzi and A. Chiozzotto, Exper. *11*, 140 (1955).

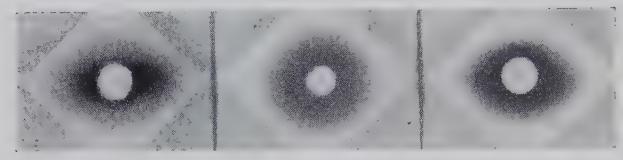
[5] M. J. Dallemagne, J. Physiol. *43*, 425 (1951).

[6] R. Klement and G. Trömel, Hoppe-Seylers Z. physiol. Chem. *213*, 263 (1932); Klin. Wschr. *12*, 292 (1933). – E. Brandenberger and H. R. Schinz, Helv. med. Acta, Suppl. 16, *12* (1945).

[7] J. B. Finean, J. sci. Instr. *30*, 60 (1953).

[8] J. B. Finean and A. Engström have observed that with a system of the type existing in the bone tissue, the use of Ni-filtered radiation (CuKα) does not entail more substantial errors than the use of monochromatic radiation.

aligned in the direction of the longitudinal axis of the bone. The diameters of the ellipsoids calculated by applying Guinier's theory come very close to the results obtained for the human bone by Engström and Finean. The short diameter is about 70 Å and the long one about 200 Å.



a *b* *c*

Still in agreement with the results of Engström and Finean, the low-angle scatter from the longitudinal sections of bone without ossein is much more symmetrical (Fig. *c*) than that obtained from untreated bone. This feature suggests that the shape and dimensions of the scattering elements are changed. In fact the short diameter of the ellipsoids is about 80 Å, while the long diameter is reduced to about 130 Å. In addition, the intensity of diffraction seems greatly increased by the removal of ossein.

The results provide the basis for the arguments in the following discussion.

The low-angle X-ray scatter might be interpreted either in terms of the *holes* irregularly placed inside a homogeneous body or in terms of a *particle scatter*, when inter-particle interference is not taken into consideration. In the general equation adopted by Guinier in treating the scatter from such systems, the intensity of the scattered radiation appears as a function of the square of the difference of the electron densities pertaining to the single components responsible for the scatter (particles and surrounding medium or homogeneous body and holes). Such a condition does not enable us to establish, a priori, to which of the two aforesaid systems a low-angle scatter is related. In this respect the choice of the system is suggested by evidence derived from the data of other investigations.

The identity of the low-angle X-ray diffraction pattern pertaining, respectively, to the bone, collagen tissue[9] and calcified collagen[10] does not permit the acceptance of the theory that the scattering units pertain to inorganic particles, these latter being completely missing in collagen tissue. Therefore, the scattering units must be considered as related to ellipsoidal entities (200 × 70 Å) pertaining to the collagen and oriented in parallel. In bone and calcified collagen, the calcium salts, enclosing the organic ellipsoids, increase the difference in electronic density between the same organic particles of collagen and the surrounding medium.

This interpretation of the low-angle X-ray scatter from bone tissue in terms of holes (containing the organic particles) irregularly placed inside a homogeneous body (the inorganic bone fraction) is also supported by the following arguments:

(a) Schmidt, 20 years ago, studying the form birefringence of the bone, furnished evidence that the inorganic fraction should most likely be considered as a homogeneous body with very fine holes occupied by the micelles belonging to the ground substance[11].

(b) A bone system resulting from inorganic particles or crystallites, dispersed in the ossein, according to Engström and Finean, could not exist when ossein had been removed, since the particles would break down into an "incoherent powder". On the contrary, bone without ossein exists as a coherent structure, though its resistance is greatly decreased. In addition, the polarizing microscope and the low- and the wide-angle X-ray diffraction patterns demonstrate that the structural orientation of the inorganic fraction is unmodified. Finally, the form birefringence curve can be recorded from the bone without ossein[12].

(c) Electron microscopical investigations[13] have confirmed the results of Schmidt's investigations. According to Ascenzi and Chiozzotto, the maximal diameter of the holes ranges from 200 to 250 Å. According to Barbour, the diameter of the same structures ranges from a minimum of 62 Å to a maximum of 225 Å.

(d) And lastly, it is very important to remark that the change observed in the low-angle scatter of the bone from which ossein has been removed appears as new evidence of the necessity to interpret the low-angle scatter of the bone as due to organic ellipsoidal particles enclosed in the holes circumscribed by the inorganic fraction. Indeed, such a change is neither related to the orientation nor the physical state of the inorganic crystallites, both conditions being unmodified by Gabriel's treatment, according to the X-ray diffraction patterns. Therefore it is more plausible to maintain that in the homogeneous body pertaining to the inorganic bone fraction, the removal of the ground-substance from the holes results in an arrangement of the material delimiting the same holes. This view finds ready support in the existence of the chemical bonds between ossein and the inorganic bone fraction[14].

Conclusions.— This array of arguments supports the view that the inorganic bone fraction may probably be regarded as a homogeneous body with holes occupied by the micelles of the organic substance. The holes correspond to ellipsoids of revolution and are aligned with the axis of the bone. The present interpretation appears to be in agreement with the properties of the bone and not in contradiction to the knowledge derived from the wide-angle diffraction pattern. This last corresponds to the crystalline units. From their aggregation derives the homogeneous body with holes responsible for the low-angle X-ray diffraction pattern.

V. Caglioti, A. Ascenzi and A. Santoro

Istituto di Chimica Generale ed Inorganica e Istituto di Anatomia Patologica, Università di Roma, November 30, 1955.

Riassunto

Gli Autori dimostrano che lo spettro di diffrazione a basso angolo del tessuto osseo è determinato da micelle elissoidali (elissoidi di rivoluzione) delle dimensioni di 200 × 70 Å, pertinenti all'osseina e accolte in incavi delimitati dalla frazione inorganica.

[9] A. C. T. North, P. M. Cowan, and J. T. Randall, Nature *174*, 1142 (1954).

[10] A. Engström and J. B. Finean in: *Nature and structure of collagen* (Discussion E) (London 1953), p. 152.

[11] W. J. Schmidt, Ber. oberhess. Ges. Nat. u. Heilk., Naturw. Abt., Giessen *15*, 219 (1933).

[12] M. J. Dallemagne and J. Melon, J. Washington Acad. Sci. *36*, 181 (1946). – A. Ascenzi, Science *112*, 84 (1950).

[13] A. Ascenzi and A. Chiozzotto, Exper. *11*, 140 (1950). – E. P. Barbour, Amer. J. phys. Anthrop. *8*, 315 (1950).

[14] V. Caglioti, A. Ascenzi, and M. Scrocco, Exper. *10*, 371 (1954).

Cholinesterase in the Development of *Ciona intestinalis* (Ascidia)

Research carried out in this laboratory on the developing Ascidian egg has determined the localization and time of appearance of various enzymes. This work has demonstrated that certain enzymes (cytochrome-oxidase[1]; succino-dehydrogenase[2]) are present from the very beginning and are localized very early, while others (phosphatase[3]; dopa-oxidase[4]) appear rather late in the development.

The *Ciona* egg has a very rapid development; at 20°C an actively swimming larva is obtained in less than 24 h. This larva has, in respect to the adult, a rather complex structure; above all possessing a nervous system with brain and spinal cord and powerful tail muscles. After the larva becomes attached, it enters into metamorphosis, the tail atrophies and the muscular cells are reabsorbed. The presence of well-developed nervous and muscular systems during a brief period of larval development, and their rapid disappearance, give rise to various questions: (a) whether or not cholinesterase is present, (b) the time of its possible appearance, (c) its location. At present, comparative studies on this enzyme in the development are lacking; such studies should lead to the solution of many problems as yet unanswered.

In our work, we have used eggs of *Ciona intestinalis*, *Phallusia mamillata* and *Clavelina lepadiformis*; however, the data here presented refer exclusively to the Ciona eggs. With the other two species, different results were obtained.

The histochemical technique of Koelle, as modified by Gomori[5], was used to indicate the presence of cholinesterase. The eggs at various stages of development were fixed for a few seconds in 80% ethanol; they were incubated *in toto* at 37°C for 1 h, with acetylthiocholine as a substrate. The following results were obtained:

(1) In the undivided egg, and in the first stages of development, there are no traces of cholinesterase.

(2) It appears for the first time only at the formation of the neurula.

(3) At this stage the cholinesterase is localized in 2 masses of presumptive muscular cells which are situated laterally and posteriorly in the embryo. No other area contains cholinesterase; particularly, it is completely lacking in the neural plate.

(4) The cholinesterase remains localized exclusively in the muscle cells during all the development until the end of the larval stage. In the Figures 1–4, embryos at successive stages of development are shown: the reaction appears in the muscle cells which are not yet differentiated. In the actively moving larva (having pigmented sensory organs), the reaction occurs in the muscle cells which are already elongated and differentiated (Fig. 5). On the other hand, there is no trace of cholinesterase in the brain and spinal cord.

(5) In the larva in metamorphosis, in which the tail has just become reabsorbed, the cholinesterase appears localized in the posterior region (Fig. 6). This is explained by the fact that, during the reabsorption of the tail, the muscle cells migrate to this portion of the trunk.

(6) In the metamorphosized larva having 2 siphons, there is no more cholinesterase.

(7) The treatment of the developing egg with eserine (0·005% solution) or with neostigmine (0·01% solution) blocks the activity of the enzyme. The treatment with di-isopropylfluorophosphate (0·002%) does not cause any inhibition.

Fig. 1–6. – Reaction of cholinesterase in *Ciona intestinalis*; incubation in acetylthiocholine for 1 h at 37°C. – 1. – Neurula. 2. – Advanced neurula. 3. – Young tail bud. 4. – Tail bud, side view. 4. – Swimming larva. 6. – Larva in metamorphosis.

On the basis of the results, it can be stated that the enzyme considered here corresponds to the "specific" cholinesterase.

Conclusions.– In the Ciona egg cholinesterase appears at a rather late stage of development (neurula stage) and is localized exclusively and permanently in the muscular territory. It appears before the differentiation and functioning of the muscles. It is yet to be investigated whe-

[1] G. Reverberi, Exper. *12*, 55 (1956).
[2] V. Mancuso, Rend. Ist. sup. Sanità *15*, 265 (1952).
[3] A. Minganti, Pubbl. Staz. zool. Napoli *25*, 9 (1954).
[4] A. Minganti, Pubbl. Staz. zool. Napoli *23*, 52 (1951).
[5] G. Gomori, *Microscopic Histochemistry* (Chicago Univ. Press, 1952), p. 210.

ther the quantity or activity increases with the muscular differentiation. The cholinesterase disappears entirely in the larva which has undergone metamorphosis and in which the muscular system has been reabsorbed. The central nervous system does not show the presence of cholinesterase in any stage of development. The treatment with specific inhibitors demonstrates that the enzyme described corresponds to specific cholinesterase or acetylcholinesterase.

M. Durante

Zoological Institute, University, Palermo, Italy, May 24, 1956.

Riassunto

Con metodo istochimico, sono state effettuate ricerche sulla presenza, la localizzazione, e il tempo di comparsa della colinesterasi nello sviluppo di *Ciona intestinalis* (Ascidie). È stato visto che: 1) l'attività colinesterasica, negativa negli stati precoci di sviluppo, si palese durante e dopo la neurulazione; 2) il territorio in cui si localizza la reazione è esclusivamente quello muscolare; 3) esiste una relazione tra la presenza dell'enzima e la capacità funzionale dell'embrione; 4) nella reazione è interessato un solo tipo di enzima e precisamente la colinesterasi specifica o acetilcolinesterasi.

Trace Element Stimulation of Keratin (Hair) Degradation by Oral Keratinolytic Microflora[1]

The proteolysis-chelation theory explains the etiology of dental caries as two interrelated reactions occurring simultaneously on enamel: (a) microbial destruction of the organic matrix which is composed largely of keratin, and (b) loss of apatite through dissolution by organic chelators some of which originate as matrix breakdown products[2]. This theory was derived in part from consideration of numerous systems throughout nature where ordinarily insoluble inorganic materials are mobilized and transported as soluble sequestration complexes[3]. It was also suggested by the Ca activation and stabilization of enzymes and other proteins, the role of Ca in proteolysis, and the ability of proteins and their derivatives to form water-soluble chelate complexes with alkaline earths[4]. The fact that $CaCO_3$ and $Ca_3(PO_4)_2$ stimulate metabolism of oral keratin-digesting microflora[2] motivated the present studies to determine whether Ca, PO_4, and other trace elements influence keratinolysis, and whether this proteolysis dissolves enamel apatite.

Experimental.—The method of cell production, conditions for Warburg respirometry, preparation of enamel and hair, and composition of the trace element supplement have already been reported[2]. Finely cut human hair was a convenient source of keratin since it is impractical to obtain purified yet undenatured enamel keratin in adequate amounts.

Results.—Both autorespiration and hair oxidation were stimulated by $CaCl_2$ (Table). The complete trace element supplement depressed autorespiration but accelerated O_2 uptake with keratin. Even without Ca and PO_4, the supplement increased O_2 consumption with hair. Thus other elements may participate in the catabolism of this material. The maximum O_2 uptake observed with the Ca-free supplement cannot mean that Ca inhibited since $CaCl_2$ enhanced the oxidation, but probably reflects trace metal unbalance or ion antagonism[5]. The enamel supplied sufficient trace elements to stimulate hair catabolism, but not enough organic matter to in-

[1] This investigation was supported in part by a research grant D-182(C) from the National Institute of Dental Research of the National Institutes of Health, United States Public Health Service.

[2] A. Schatz and J. J. Martin, N. Y. St. dent. J. *21*, 367 (1955). – A. Schatz, K. E. Karlson, and J. J. Martin, N. Y. St. dent. J. *21*, 438 (1955). – A. Schatz, J. J. Martin, K. E. Karlson, and V. Schatz, N. Y. St. dent. J. *22*, 161 (1956).

[3] A. Schatz, N. D. Cheronis, V. Schatz, and G. S. Trelawny, Proc. Penn. Acad. Sci. *28*, 44 (1954). – J. J. Martin, H. D. Isenberg, V. Schatz, G. S. Trelawny, and A. Schatz, Euclides *14*, 311 (1954). – A. Schatz, Umschau *24*, 746 (1955).

[4] F. R. N. Gurd (editor), *Chemical Specificity in Biological Interactions* (Academic Press Inc., New York 1954).

[5] S. H. Hutner, L. Provasoli, A. Schatz, and C. P. Haskins, Proc. Amer. philos. Soc. *94*, 152 (1950).

Trace element stimulation of keratin (hair) degradation by oral keratinolytic microorganisms

Distilled H_2O cell suspension supplemented with	400 min Warburg experiment			
	Autorespiration (no hair present)		100·0 mg hair added to each vessel	
	μl O_2 uptake	μl O_2 stimulation over control due to trace elements	μl O_2 uptake	μl O_2 stimulation over control due to trace elements
Ca as $CaCl_2$ (5·0 mg%)	78	30	832	95
Trace element supplement	37	−11	1025	288
Trace element supplement minus Ca and phosphate	55	7	850	113
Trace element supplement minus Ca	58	10	1042	305
Human enamel as source of trace elements (50·0 mg added to each vessel)	47	− 1	950	213
No minerals added (controls)	48		737	

pH of cell suspension initially adjusted to 7·0; KOH in each center well; 30°C. Trace element supplement includes Na, K, Ca, Mg, Mn, Zn, Mo, Fe, Co, Cu, P, S, and Cl in the same concentrations employed in the basal culture medium for growing keratinolytic microorganisms[2]. The data have not been corrected by subtracting autorespiration.

crease O_2 uptake over the hair-free control. To accomplish this, at least 100·0 mg of enamel were required. Thus, in these experiments involving keratin degradation, trace elements increased O_2 consumption to a greater extent than is attributable to stimulation of autorespiration alone.

These observations agree with other experiments (to be published) in which oral proteolytic microflora catabolized pulverized Ca^{45}-rat bone, as a result of which the pH rose from around 7·0 up to 8·0 and appreciable amounts of Ca^{45} passed into solution. The most likely mechanism whereby bone and enamel minerals dissolved under the conditions in these experiments is by the release of chelating agents as degradation products of organic matrix constituents.

Conclusions.—These studies indicate that enamel minerals stimulate keratinolysis, and keratinolysis contributes to dissolution of enamel apatite. Consequently, the simultaneous disintegration of organic and inorganic components of enamel during formation of dental caries appears more likely than an independent attack on either phase alone. In terms of comparative biochemistry, the proteolysis-chelation theory views tooth decay as due to essentially the same mechanism responsible for pathological bone resorption, normal deciduous tooth root resorption, dental cavities in marine mammals, lobster and mollusc diseases where the calcareous shells demineralize, and solubilization of rock phosphate (i.e. apatite) fertilizer in neutral and alkaline soils[6]. In these systems, acid is not the sole or major factor causing solubilization or decalcification, but chelation seems to be the common denominator. Organic chelators of microbial origin which dissolve calcium phosphates, even under neutral and alkaline conditions, may be useful biological reagents with which to attack such problems as the nature of the bonding between the organic and inorganic components of calcified body structures, changes with age, and the influence of fluoride.

A. Schatz, K. E. Karlson, and J. J. Martin

Research Laboratory, National Agricultural College, Doylestown, Bucks County, Pennsylvania, March 3, 1956.

Zusammenfassung

Nach der Proteolyse-Chelationstheorie der Zahnkaries werden organische und anorganische Bestandteile des Zahnschmelzes mehr oder weniger gleichzeitig abgebaut. Die Ergebnisse dieser Untersuchungen zeigen, dass 1. mineralische Bestandteile des Schmelzes die mikrobiologische Zersetzung des Keratins fördern und dass 2. die enzymatische Zersetzung des Keratins Stoffe entwickelt, welche die Apatitauflösung mittels Chelation fördern.

[6] A. Schatz and J. J. Martin, N. Y. St. dent. J. *21*, 367 (1955). – A. Schatz, K. E. Karlson, and J. J. Martin, N. Y. St. dent. J. *21*, 438 (1955). – A. Schatz, J. J. Martin, K. E. Karlson, and V. Schatz, N. Y. St. dent. J. *22*, 161 (1956). – A. Schatz, N. D. Cheronis, V. Schatz, and G. S. Trelawny, Proc. Penn. Acad. Sci. *28*, 44 (1954). – J. J. Martin, H. D. Isenberg, V. Schatz, G. S. Trelawny, and A. Schatz, Euclides *14*, 311 (1954). – A. Schatz, Umschau *24*, 746 (1955).

The Biometry of the Cirripede, *Chthamalus stellatus* (Poli)

Data have recently been given by Tenerelli[1] on the sizes of *Chthamalus stellatus stellatus* (Poli) and *C. stellatus depressus* (Poli) together with a discussion of the status of the 2 forms. These results are of considerable interest and would seem to warrant a more detailed statistical treatment.

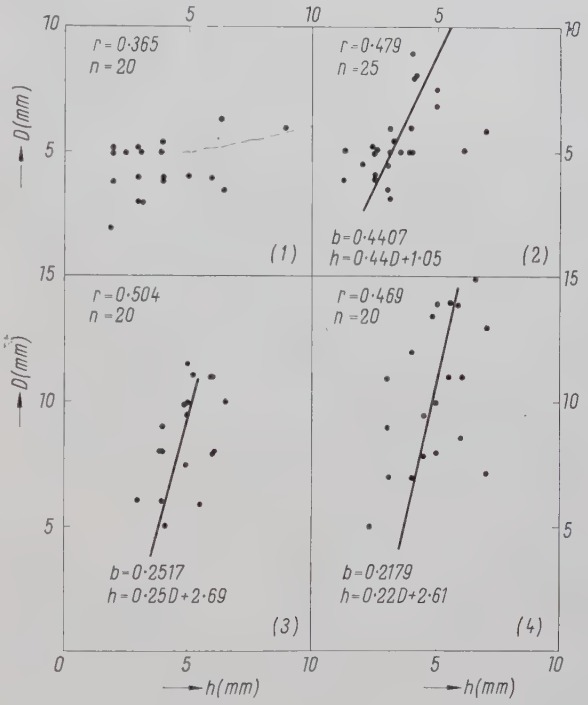


Fig. 1.–Plot of diameter (D) against height (h) for *Chthamalus stellatus stellatus* (Poli). Data from Tenerelli: (1) Zona inferiore di bassa marea. (2) Zona intercotidale, vicino alla linea superiore di alta marea. (3) 40–60 cm sulla linea di alta marea. (4) 150–180 cm sulla linea superiore di alta marea. Correlation coefficients and regression lines are also shown.

Measurements are given of the height and basal diameter of individuals of both varieties collected at several levels of the intertidal zone. Tenerelli points out that the form *depressus* reaches greater dimensions than *stellatus* but he does not distinguish between the different year classes. He further shows that the ratio of the height to the diameter is different in the 2 forms. It is more instructional to consider the relation between the heights of the individuals in respect to the diameter, at the different levels and in particular to determine whether this relation varies with the level in either of the forms; any such variation could indicate the effect of environment on the growth form of the animals.

All the data, height against diameter, have been plotted in the Figures 1 and 2. With the exception of form *stellatus* at the lowest level (zona inferiore di bassa marea) and the form *depressus* at the highest level (180–260 cm sulla linea superiore di alta marea) there is a significant correlation between the 2 dimensions. In the former case the lack of correlation may be due to the

[1] V. Tenerelli, Atti Accad. Gioenia Sci. nat. Catania [6] *9*, 92 (1952–53).

effect of crowding by other organisms since there is little change in diameter with increasing height. In the latter case the opposite is true, there is little change in height as the diameter increases; with these large and presumably old barnacles at a very high and exposed level this would seem to be the result of abrasion. With these exceptions the appropriate regression lines of heights on diameter have been calculated and are also shown in the Figures.

It is a simple matter to test whether a set of samples can be considered to be drawn from a population having the same regression. The difference between the residual sums of squares of the combined regression line and those of the separate lines divided by the difference of the degrees of freedom is an independent estimate of the variance which may be compared with the residual variance from the separate regression lines.

Applying this test it is found that all the samples from the various levels of each form considered separately have a common regression line, i.e. for each form the relation between diameter and height is the same at all levels. However, when the common regression lines for the 2 forms (pooling all the individual results) are compared they are found to be significantly different.

A common regression line for all levels for either form suggests that the relation is little influenced by environment and together with the significant difference between the common regression strengthens Tenerelli's separation of the species into 2 distinct varieties.

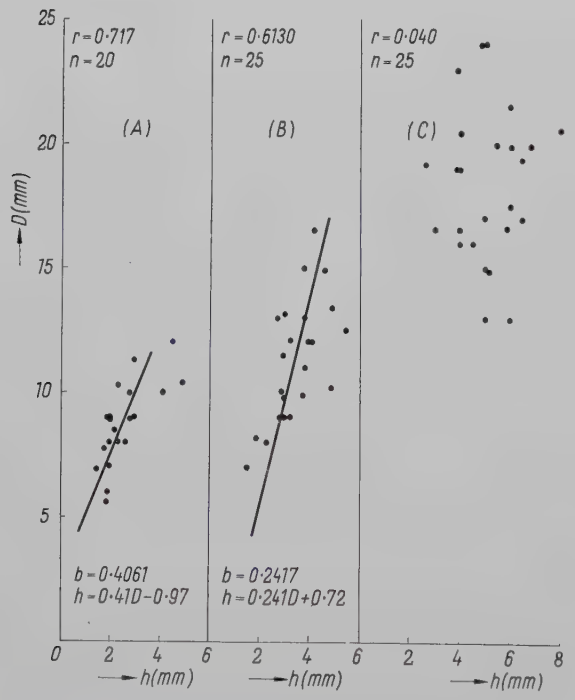


Fig. 2.–Plot of diameter (D) against height (h) for *Chthamalus stellatus depressus* (Poli). Data from Tenerelli: (A) Zona intercotidale, vicino alla linea superiore di alta marea. (B) 40–50 cm sulla linea superiore di alta marea. (C) 180–260 cm sulla linea superiore di alta marea. Correlation and regression lines are shown.

It is also of interest to note that, whilst the pooled regression lines are significantly different, this difference is one of position and not slope (Fig. 3); the rate of change of height with diameter is not significantly different. It appears from Figure 3 that the relation in the case of the form *depressus* is linear throughout life since production of the regression line gives values

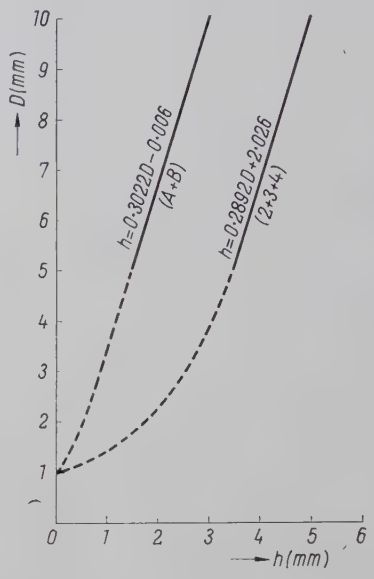


Fig. 3.–The common regression lines for the 2 forms *stellatus* (*2*, *3*, *4*) and *depressus* (*A*, *B*). Regression lines produced to show necessary change of slope in *stellatus*.

roughly equivalent to those expected in the newly settled animal; in the case of the form *stellatus* the same argument suggests that a change in the diameter-height relation takes place between settlement and a value of 5 mm for the diameter.

H. Barnes

The Marine Station, Millport, Scotland, November 7, 1955.

Résumé

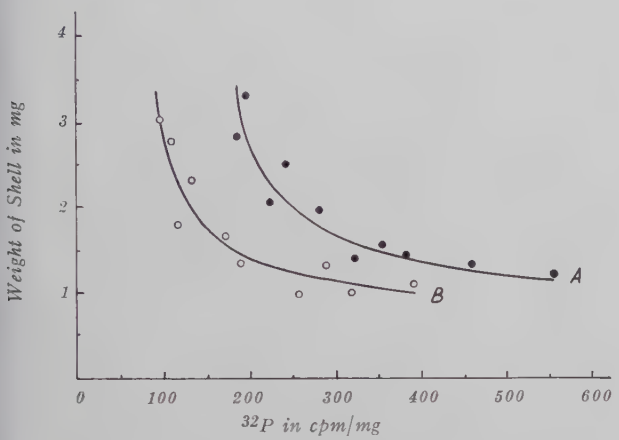
Les données de Tenerelli sur les dimensions (hauteur et diamètre basal) de *Chthamalus stellatus stellatus* (Poli) et *C. stellatus depressus* (Poli) sont analysées ici par des méthodes statistiques. On peut constater une ligne de régression commune à tous les échantillons de chaque sous-espèce, pris à plusieurs niveaux. Les lignes de régression pour les deux sous-espèces en question offrent des différences significatives. Leur croissance diffère également.

The Exchange of Phosphorus in Shells of the Aquatic Snail *Physa acuta*

Many authors have been able to demonstrate by using radioactive isotopes the exchange of phosphorus and calcium in bones of higher animals. It therefore was of some interest to find out whether similar exchanges of important biogenic elements occur in the shells of some mollusca. Concerning generally the problem of basal metabolism of phosphorus in the aquatic snail *Physa acuta*[1] cultivated in our laboratory, we performed some experiments where finally a relatively intensive

[1] These studies were part of our research on the mode of action of molluscacidal substances on the snail's metabolism.

exchange of phosphorus in the shells of these mollusca could be determined. It was mainly the P-metabolism of growing animals which was studied in our experiments. Thus we found, e.g. with the type *Physa acuta*, that approximately 50% of the accepted ^{32}P-isotope changes in its shell in the course of 5 days. A rather peculiar observation was made that the percentage of the ^{32}P exchanged in the shells was the same, even though individuals of different shell-weight, and thus of different age, were used. At the same time the absolute quantity of the bound ^{32}P was indirectly proportional to the shell-weight, i.e. the ligther the shell the more ^{32}P was bound by it. This phenomenon can easily be explained by the more intensive metabolic activity of young growing animals. The following Graph shows the uptake of ^{32}P in 5 days (*A*), and at the same time its exchange during the following 5 days (*B*), in shells of the *Physa acuta*.



cpm/mg = counts per min per mg weight of snail-shell.
Controls performed with shells containing no animal did not show any significant uptake of radiophosphorus during 5 days.

Methods.— 20 young mollusca of different size were grown for 5 days in a medium (100 ml of tap-water from which Cl_2 were eliminated) containing 1 μc/ml ^{32}P (obtained by neutralization of H_3PO_4 by NaOH). After 5 days all the animals were taken out of the medium and washed briefly with distilled water 10 times until the distilled water showed no more radioactivity. 10 of them were killed in boiling distilled water, the bodies were shelled and the shells, after washing 3 times with distilled water, dried at 60° for 24 h (the part of shells designated with *A* in the Graph). The other 10 animals, after 1 h in pure tap-water, were transferred to the same jar under the conditions described above, but without any ^{32}P. After 5 days, these 10 snails were killed and dried in the same manner as is described above (the *B* group in the Graph). Then the shells of both groups were weighed and ground to powder, and the activity of 1 mg of the shell-powder was estimated with a G.M. counter.

Because it is well known that phosphorus does not belong to the basal building material of the mollusca-shell, our experiments seem to confirm the old conception of phosphorus being the agent which transfers calcium to the shells[1]. This conception supposes in principle that calcium is transferred to the organic layers of the shell, perhaps as soluble calcium diphosphate, and there this crystallized compound is readily changed into calcium carbonate, the real building matter of the shell. Phosphate is then brought back to the normal snail metabolism. If this conception were right, then the whole calcium transfer by means of phosphorus would be far more complicated than is usually supposed in the ossification of mammals[2].

M. Polster

Institute of Microbiology, Faculty of Medicine, University of Brno, Czechoslovakia, May 25, 1956.

Zusammenfassung

In Schalen von wachsenden Wasserschnecken, *Physa acuta*, wurde binnen 5 Tagen 50 % Austausch des Radiophosphors ^{32}P beobachtet. Auch wurde festgestellt, dass das absolute Quantum des ^{32}P, der binnen 5 Tagen aufgenommen wird, indirekt proportional dem Schalengewicht ist, das heisst, je schwerer die Schale ist, desto mehr ^{32}P wird aufgenommen.

[1] H. Simroth and M. Hoffmann, in: *Dr. H. G. Bronn's Klassen und Ordnungen des Tierreiches*, III. Bd. (Mollusca), II. Abt., 2. Buch (1928), p. 192.

[2] Th. Bersin, *Kurzes Lehrbuch der Enzymologie*, 4. Aufl. (Akad. Verlagsges., Leipzig 1954), p. 297.

Streptomycin and Endocrine System

In a previous work[1] we published data that streptomycin and inositol (which forms a part of the streptomycin molecule), injected into healthy rabbits produces a rise in the neutral blood fat content; and the same is seen, though in a lesser degree, in persons treated with the therapeutic dose of streptomycin.

We believed that it is mainly the function of one or several members of the hypophyseal-hypothalamic-adrenal system which should be considered as probable cause of the changes in the fat metabolism which we established. This assumption can be sustained by several clinical observations described in the complications of streptomycin treatment as the manifestation of hypertrichosis (Fonó[2]), the appearance of striae atrophicae (Boquien and others[3]), hyperglycaemia, acne, retention of sodium, the decrease of the number of circulating eosinophils and lymphocytes (Barnard[4]), signs which may also develop as clinical features of different endocrine disorders.

Recently Károlyházi[5] in our clinic found that the diabetic state in alloxan induced rats was significantly deteriorated by the administration of streptomycin.

To elucidate the mechanism of this action of streptomycin, systematic researches were begun with the investigation of the possible changes in the function of the anterior pituitary.

The first experiments were performed on 12 healthy rabbits kept on the same diet, and the changes of the dextrose, dextrose + insulin, dextrose + insulin + vitamin B_1 and finally dextrose + vitamin B_1 tolerance tests were determined before and after treatment. The resul-

[1] L. Mosonyi *et al.*, Lancet *2*, 81 (1951); Wien. Z. inn. Med. *33*, 384 (1952).
[2] R. Fonó, Orv. Lapja *5*, 515 (1949).
[3] Y. D. Boquien *et al.*, Bull. Soc. méd. Hôp. Paris *1948*, 852.
[4] R. Barnard, Lancet *1*, 612 (1952).
[5] Gy. Károlyházi, Orv. Hetil. *94*, 34 (1953).

Rabbit No.	Steroid excretion (*A* 17-ketosteroid, *B* 11-oxysteroid) mg in 24 h							
	before				after			
	Streptomycin treatment							
			Response to ACTH				Response to ACTH	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	0·7	0·14			1·15	0·64	0·95	0·70
2	0·55	0·35			0·61	0·36	0·99	0·76
3	0·61	0·37			0·61	0·45	0·69	0·45
5	0·65	0·085			0·16	0·13	0·3	0·30
6	0·9	0·21	2·0	1·23	2·4	0·26	1·8	0·36
7	1·35	0·38	2·0	0·75	1·15	0·1	0·95	0·15
8	–	0·45	2·0	1·1	2·0	0·18	1·2	0·23
9	1·7	0·4	2·3	1·25	0·76	0·08	0·9	0·18
10	4·1	0·66	4·1	0·89	1·05	0·15	2·15	0·17
11	–	0·5	–	0·9	1·9	0·29	0·5	0·22
Mean (Rabbit No. 10 omitted)	1·05 ± 0·13	0·37 ± 0·06 Sign. Diff. = 6·6	2·1	1·03 ± 0·08 Sign. Diff. = 6·6		0·28 ± 0·05		0·37 ± 0·08

ting curves were evaluated through planimetric comparison. The experiments were carried out using streptomycin and inositol separately in the two series.

Between the two series of tests, for a period of 3 weeks we daily injected intramuscularly 15 mg streptomycin or 6·7 mg inositol (equimolecular with 15 mg streptomycin), per kg body weight to both groups of rabbits. There were no complications at all and the animals tolerated the injections well. Similarly to our earlier findings, a significant rise in the neutral blood fat content was found in the majority of the rabbits.

It could be established from the 4 series of experiments that there is no definite change in the secretion of the carbohydrate regulating factors of the anterior pituitary during streptomycin treatment. The same evidence could be concluded concerning the function of the STH and ACTH. 40 young male albino rats were injected throughout 6 weeks with 15 mg streptomycin per kg body weight daily. Neither in the length of the tails, nor in the body weight, nor in that of the liver and adrenals could any significant difference be detected between streptomycin-treated and control animals. It would be illogical, therefore, to maintain that the anterior pituitary plays the chief role in the "general effect" of streptomycin.

Recently male rabbits were injected in the same manner with streptomycin and determinations of the blood sugar, potassium, sodium, neutral fat and cholesterol content of the serum were performed simultaneously with measurements of the 24 h 17-ketosteroid and 11-oxysteroid excretion before and after the injections. It could be concluded that there is a significant change in the adrenal cortex activity following streptomycin treatment. The body weight rose in 8 of the 11 rabbits, the serum potassium content fell, the serum sodium level rose in all cases and so did the blood sugar, the neutral fat and also the cholesterol content of the serum in the majority of the animals. The greatest and most uniform change could be observed in the response to ACTH administration (Table).

As Clayton and Hammant[6] stated, guinea pigs respond to ACTH regularly with 1 mg elevation of the 24 h excretion.

The 17-ketosteroid excretion usually responds with lesser increase on the administration of ACTH, than that of the 11-oxysteroid, though the effect of ACTH on cholesterol favours the production of pregnenolone and the conditions to produce 17-ketosteroids (Renold[7]).

The excretions prior to the antibiotic treatment differ principally from those after the administration of the antibiotic.

(a) Before the treatment the response to ACTH is normal in the excretion of 17-ketosteroids as well as in that of the 11-oxysteroids.

(b) After the prolonged treatment the response in the excretion of 17-ketosteroids shows an increase in 5 cases, in 5 a fall.

(c) After the streptomycin treatment the rise in the excretion of 11-oxysteroid in response to ACTH is lessened.

The excretion of cortical steroids follows an intra-adrenal enzymatic activity (Bishop[8]), the impairment of which may result in an unusual relation between the two groups of hormones. In another series of experiments on *in vivo* perfusated dog adrenals[9], we found, indeed, that the acutely administered streptomycin has an impairing action on these processes. This often results in a lessened secretion of both hormones, in other cases there is a reciprocal relation between the two hormones: the production of one hormone is raised, of the other diminished.

From the above experiments, it can be concluded that the prolonged administration of the antibiotic may result in a similar impairment. This is manifested in the following directions: (1) the increase in electrolyte retaining capacity, (2) the irregular response to ACTH in the 17-ketosteroid excretion, (3) the lessened degree of response in the 11-oxysteroid production.

These results cannot be invalidated by the fact that there is no constancy in the 11-oxysteroid excretion of different individuals, because there is no reason to infer the same changes in the ACTH responsiveness.

The two types of responses explain the variations in the clinical signs referred to. It is not necessary that any clinical manifestation whatever be present. However, if the excretion of androgenic hormones prevail, hirsutism may appear; if the antibiotic interferes with the produc-

[6] B. E. Clayton and I. E. Hammant, Nature *176*, 401 (1955).

[7] A. E. Renold *et al.*, J. clin. Endocrin. *12*, 763 (1952).

[8] Pm. F. Bishop, *Recent Advances in Endocrinology* (Cameron) (J. A. Churchill Ltd., London 1954).

[9] L. Mosonyi, M. Mincsev, L. Pollák, and E. Németh (in press).

tion of androgenes, the resulting higher (or relatively higher) glucocorticoid production may give rise to Cushingoid signs.

Observations in human beings are in concordance with the above data.

A 30 years old woman was treated abroad against her febrile illness with streptomycin. The treatment lasted several weeks and the patient got approximately 30 g of the antibiotic. Following the cure hypertrichosis developed. No endocrinological abnormality could be detected for this phenomenon, save the elevated 17-ketosteroid excretion (13 mg). The 11-oxysteroid excretion amounted to 0·76 mg. The mean, normal value in 17-ketosteroid excretion of our female patients is 10·85 mg. According to this the value lies in the upper range. In contrast to which, the 11-oxysteroid excretion was unusually low.

Finally the impairment of the enzymatic processes of the adrenal cortex may result, as DEBRÉ[10] states, in the hyperactivity of the anterior pituitary. This may manifest itself in deterioration of the alloxan-induced diabetes in rats, as experienced by KÁROLYHÁZI.

The clinical and the experimental evidence leads to the conception that interference with the adrenocortical enzyme function is responsible for the endocrine signs in streptomycin therapy.

L. MOSONYI, L. POLLÁK, R. ZULIK, and GY. KÁROLYHÁZI

II. Medical Clinic of the University of Budapest, Hungary, April 16, 1956.

Zusammenfassung

Auf Grund klinischer Erfahrungen, nach welchen anlässlich einer Streptomycintherapie verschiedene endokrine Störungen beobachtet werden konnten, wurden zur Klärung derselben Tierexperimente durchgeführt. Die Versuchsergebnisse deuten auf eine funktionelle Störung der Nebennierenrindenenzyme hin, hervorgerufen durch die Einwirkung des Antibiotikums, wobei die verschiedenen klinischen Symptome als Manifestationen der Verschiebung der normalen Hormonverhältnisse aufgefasst werden.

[10] R. DEBRÉ *et al.*, Pr. méd. *85*, 583 (1954).

Diagnostic Test for Cancer

During an investigation of possible chemical changes in the spleen of rats following weekly injections of chemical carcinogens over a seven weeks period, it was noted that the lipid fraction of the spleen of injected rats had a distinctly darker yellow color than did similar fractions of normal rat spleen. The lipid fraction from a transplanted rat sarcoma had the same color as that of the spleen following treatment of rats with 1, 2, 5, 2-dibenzanthracene (DBA).

This observation leaving to attempts of characterization of the phospholipids from different tumors and from spleen after treatment of rats with various carcinogens and non-carcinogens, was described by HAKIM[1].

In addition to immunological studies by chromatographic and paper ionophoretic techniques, a modification of the Feulgen reaction was found to be useful in the identification and characterization of the new phospholipids induced by chemical carcinogens in the spleen. These new phospholipids could also be revealed at the same time in serum.

This new abnormal acetat-lipid, could be tested for in the serum of tumor carrying animals, and recently it was revealed in all *human cancer cases* tested by 2 techniques, in the following way:

Modified Feulgen Reaction.— To 500 µl of the serum the following reagents are added in the order given:

10 µl of 0·5 % Ascorbic acid,
20 µl of 0·5 % Thiamine HCl,
100 µl of 95 % alcohol,
150 µl of 10 % KOH
100 µl of 0·5 % $CuSO_4$
120 µl of 0·02% fuchsin (basic fuchsin).

The mixture is shaken after each addition. The color is noted between 15–30 min in the sun light.

Normal sera gave a *red* color, while sera from tumor bearing animals, and from *human cancer cases* when tested in a similar manner gave a *purple* color.

Paper Electrophoresis.— Electrophoresis was accomplished in the LKB electrophoretic equipment, using 40 × 410 mm Schleicher and Schuell No. 2043B (120 g/m²) filter paper, or Whatman No. 1 filter paper. Veronal buffer, pH 8·6 with ionic strength 0·125, or Veronal-borate buffer (equal volumes of veronal buffer pH 8·6, ionic strength 0·060 ionic strength, and Borate buffer pH 8·6, 0·1 *M*). The test is carried at room temperature (about 18° C), with a potential of 200 V across the filter paper, and a current of 8 mA, for 24 h. After electrophoresis, the electrogram was dried and stained for proteins with bromphenol.

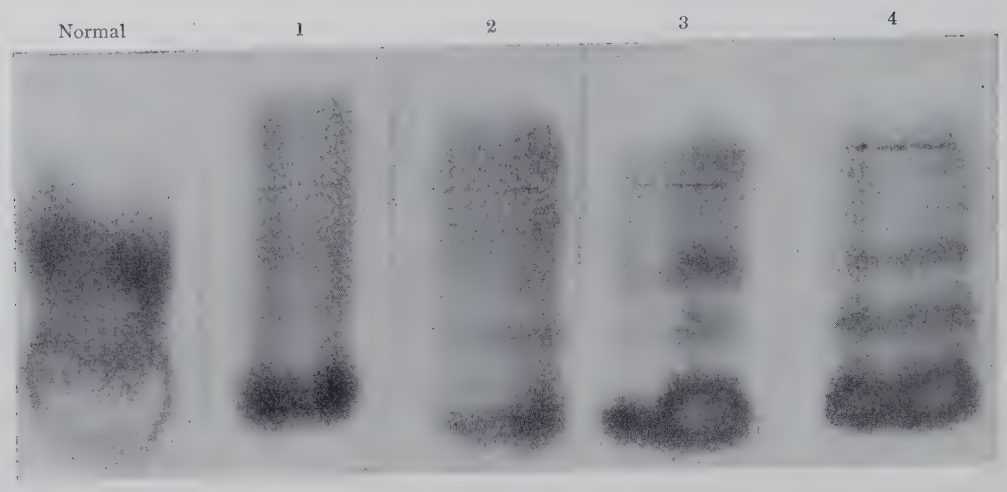
A more general survey is in progress, to determine the possible physiological conditions, on either secondary infections, or complications with cancer which might alter the above described reactions. Similar studies are in progress to determine whether fibrinogenic, or infectious disease might give a similar reaction, in non-cancer, non-normal persons.

The presence of an abnormal gamma globulin in the sera of human cancer sera is evident. Typical electrophoretic tests, presented in the joined Figure include

[1] A. A. HAKIM, Exp. Med. Surg. (in press); Proc. Amer. Ass. Cancer Res. *1956*, April 15.

Serological Reactions for Cancer

	Modified Feulgen reaction			Paper ionophoretic reaction		
	Total Number Tested	Positives	Negatives	Total Number Tested	Positives	Negatives
normal persons	100	2	98	100	100	100
known Cancer persons	75	75	0	75	75	0



Diagnostic Test for Cancer: Paper electrogram of sera obtained from the following patients, as a typical example: 1. G. B. Pleomorphic CA lung; 2. S. H. S. Squamous cell CA lung; 3. W. R. C. Epidermoid CA lung; 4. I. V. Squamous cell CA bronchus, as compared with the electrogram of a serum obtained from a normal person

sera from the following patients: (1) G.B. Pleomorphic CA lung, (2) S.H.S. Squamous cell CA lung, (3) W.R.C. Epidermoid CA lung, (4) I.V. Squamous cell CA bronchus. Compared with the electrogram of a serum obtained from a normal person.

The 2 tests are performed successively; it was noted, that normal persons, who are heavy smokers, gave a serum which reacted abnormally, and gave a purple color when tested by the modified Feulgen reaction, but on electrophoresis showed no abnormal gamma globulins.

A. A. Hakim

Medical Research Department National Children's Cardiac Hospital, Miami 34, Florida, March 24, 1956.

Résumé

1° Une modification de la réaction de Feulgen est décrite. En l'utilisant, on constate que les sérums des personnes normales prennent une couleur différente de celle des sérums des personnes cancéreuses.

2° En appliquant l'électrophorèse sur papier, avec Veronalborate tamponné, on a décelé la présence d'un gamma-globuline atypique dans les sérums des personnes cancéreuses.

Action du rayonnement X sur la croissance de la plantule d'orge

En 1954, Schwartz[1] a attiré l'attention sur un phénomène intéressant observé après l'irradiation de graines sèches de *Zea maïs* dans deux conditions expérimentales différentes. Il s'agit de modifications de croissance de plantules provoquées par diverses doses de neutrons et de rayons gamma du Co^{60}. Cet auteur a observé que, lorsqu'on augmente progressivement les doses de radiation, les hauteurs des plantules diminuent d'abord (de 75000 à 125000 r de rayonnement gamma) puis augmentent lorsque la dose atteint 125000 à 500000 r.

[1] D. Schwartz, Science *119*, 45 (1954).

I. – *Techniques.* Nous utilisons des graines d'orge (*Hordeum sativum* Jess) de la lignée 456 de Gembloux. Ces graines sèches sont irradiées par les rayons X mous: tube à fenêtre de beryllium fonctionnant sans filtre, 40 kV, 25 mA, distance focale 12 cm, champ de 5 cm de diamètre, débit environ 9000 r/min, de 50000 à 800000 r.

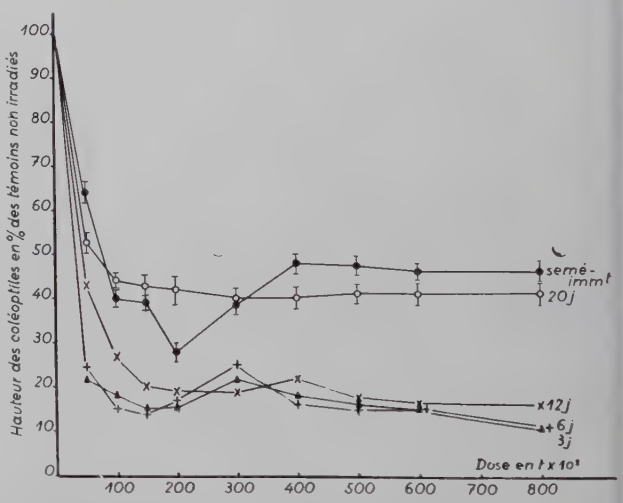


Fig. 1. Graines d'orge irradiées, mises en germination à des moments différents après l'irridation (voir texte).

Après irradiation, les graines sont semées immédiatement et après des temps de latence de 3, 6, 12 et 20 jours, dans des plateaux de 38 sur 50 cm sur une couche de 1 cm d'épaisseur d'ouate recouverte de papier filtre. Les graines sont arrosées chaque jour par 300 cm^3 d'eau distillée. Pendant la germination, la température oscille entre 20 et 21°C; l'humidité relative est de 60%; l'intensité lumineuse est de 3000 Lux; l'éclairement est de 16 h sur 24. Les hauteurs des coléoptiles sont mesurées dès leur germination.

II. – Résultats. 1° *Etude du phénomène* de Schwartz.

a) Au fur et à mesure que l'on augmente la dose de rayonnement X, les hauteurs des coléoptiles diminuent depuis la dose de 50000 r jusqu'à 200000 r.

b) Au delà de 200000 r, les hauteurs des coléoptiles augmentent jusqu'à la dose de 400000 r inclusivement

Tableau I. Longueurs en μ.

Doses en 10^3 r	Témoins	50	100	150	200	300	400	500	600	800
Longueurs en μ .	220	287	320	360	406	576	580	601	510	528
σ	$\pm$ 3,8	$\pm$ 4,6			$\pm$ 4,8			$\pm$ 10,8		$\pm$ 6,4

Tableau II. Mitoses et anomalies nucléaires dans les cellules méristématiques des coléoptiles de l'orge après 4 jours de germination de graines irradiées. Les résultats sont exprimés en pourcentage des cellules observées (environ 100).

Doses en 10^3 r	Témoins	50	100	150	200	300 à 800
Taux de mitoses	12,5	10,8	8,4	8	0,01	–
Ponts chromosomiques	–	0,2	0,4	1	–	–
Anneaux d'inversion	–	0,1	0,4	0,3	–	–
Translocations	–	0,1	0,6	1,3	–	–
Métaphases-anaphases	–	1	1,2	1,6	–	–
Fragments chromosomiques . . .	–	1,1	2,3	6	–	–
Extrachromosomes	–	3,5	5,1	5,4	–	–
Noyaux éclatés	–	5,9	10,8	12,3	13,7	–
Micronoyaux	–	1	1,5	3	4,6	–
Pycnoses	–	15	25	40	57	>90

c) A des doses supérieures à 400 000 r, les hauteurs des coléoptiles diminuent légèrement jusqu'à 800 000 r, mais restent nettement supérieures aux hauteurs mesurées après des doses d'environ 200 000 r.

Il y a donc chez l'orge un phénomène analogue à celui décrit par SCHWARTZ chez *Zea maïs*.

Si l'on envisage l'évolution de ce phénomène dans le temps, on constate qu'il n'est pas visible, le 1er et 2e jour après la germination. Il se manifeste dès le 3e jour après le début de la germination et s'accentue le 4e jour. Dans nos conditions expérimentales, l'effet est optimum le 4e jour après le début de la germination; le 5e jour et les jours suivants, il se montre pratiquement constant jusqu'à la mort des plantules.

Nous avons mesuré les longueurs de 100 cellules épidermiques des coléoptiles fixés au Carnoy et colorés au Feulgen, le 4e jour après la germination. Le Tableau I montre un accroissement continu de la longueur cellulaire jusqu'à 500 000 r.

L'estimation des taux de mitoses et d'aberrations chromosomiques est résumée dans le Tableau II.

a) Il y a diminution régulière du taux de mitoses en allant du témoin à la dose de 200 000 r.

b) Toute dose supérieure à 200 000 r produit une inhibition totale des mitoses et détermine une pycnose généralisée.

c) Il y a une augmentation progressive du taux de tous les types d'aberrations chromosomiques, en allant de la dose de 50 000 r à la dose de 150 000 r.

2° *Etude de l'influence de divers temps de latence.* (Fig.) Si, avant d'ensemencer les graines irradiées, on attend 2 jours au plus, on observe:

a) un abaissement général des courbes par rapport à la courbe tracée après ensemencement immédiat après irradiation;

b) après 20 jours de latence, la courbe reste en dessous de celle obtenue après ensemencement immédiat, sauf pour des doses allant de 100 000 à 300 000 r.

c) Le phénomène décrit par SCHWARTZ est encore perceptible après 3 jours. Il l'est moins après 6 jours; il n'est presque plus visible après 12 jours, il n'existe plus après 20 jours de latence.

d) Après 20 jours, la croissance est sensiblement plus forte par rapport aux croissances observées après des temps de latence inférieurs à 20 jours.

J. MOUTSCHEN, Z. M. BACQ et A. HERVE

Laboratoire de Morphologie végétale, de Pathologie générale et de Radiobiologie de l'Université de Liège, Laboratoire de Recherches pour la Protection des Populations civiles, Liège, le 7 mai 1956.

Summary

We have reproduced in *Hordeum* the phenomenon described by SCHWARTZ: absence of proportionality at certain dosages, between the dose of roentgen rays and the biological effect when one irradiates dry seeds and measures the growth of the coleoptile. Cytological analysis shows an enlargement of the epidermic cells of the coleoptiles up to 500·000 r and a decrease of the number of mitosis up to 200·000 r dose at which the mitotic inhibition is nearly complete.

The phenomenon described by SCHWARTZ disappears progressively if one waits 3 to 20 days between irradiation and sowing.

Relations entre les taux cétoniques du plasma et des organes

Comme suite à nos précedentes recherches sur l'inconstance des milieux intérieurs et le mécanisme des corrélations[1], nous avons étudié les rapports entre les taux cétoniques du plasma sanguin et ceux de différents organes. Les résultats de notre sondage statistique sont consignés dans le Tableau suivant:

[1] E. SCHREIDER, Biotypologie *13*, 20 (1952); Nature *171*, 339 (1953).

Tableau I

Corrélations entre les taux des corps cétoniques (dosés comme acétone) dans le plasma et dans cinq organes (Rats, mâles adultes, $n = 30$)

	1	2	3	4	5	6
1. foie	—	0,37	0,55	0,63	0,63	0,25
2. muscle strié . . .			0,00	0,71	0,77	0,18
3. poumon				0,54	0,37	0,16
4. rein					0,73	0,34
5. cerveau						0,41
6. plasma sanguin .						—

A première vue ces chiffres se trouvent en contradiction avec le schéma admis du métabolisme intermédiaire des acides gras. On estime généralement que le foie est le principal producteur des corps cétoniques, voire le seul capable de les introduire dans le circuit sanguin qui en assurerait la distribution dans l'organisme. Or, le sang donne des corrélations très faibles avec le foie comme avec les autres organes. D'une manière un peu paradoxale, il présente une corrélation à peine significative avec le cerveau qui, pour autant que l'on sache, n'utilise pas les corps cétoniques du plasma. Par contre, il y a plusieurs corrélations statistiquement valables *entre organes*.

A la réflexion, il est facile de comprendre que le «modèle» mathématique compatible avec nos chiffres ne s'oppose point au schéma accepté en physiologie. Mais nos résultats font croire que *la régulation du taux cétonique des organes ne dépend pas des variations du meme taux dans le sang*. Ces résultats peuvent être présentés aussi sous une autre forme qui éclaircit davantage la situation:

Tableau II

Analyse factorielle, méthode centroïde. Coefficients de saturation

	Facteurs		
	I	II	III
foie	0,74	0,29	0,07
muscle strié	0,68	−0,43	0,41
poumon.	0,52	0,60	0,24
rein	0,89	−0,08	0,18
cerveau	0,89	−0,31	0,19
plasma sanguin	0,42	−0,10	−0,24

Selon le premier facteur, le taux cétonique de plusieurs organes pourrait en partie obéir à une cause commune: c'est le cas du foie, du muscle, du rein, du cerveau et, dans une moindre mesure, du poumon. Le plasma sanguin ne présente pas de liaison aussi étroite avec le premier facteur, on peut même concevoir que son coefficient, dans la première colonne, soit un artefact mathématique, car au point de départ il n'y a qu'une seule corrélation significative (avec le cerveau), médiocre en valeur absolue et contredite par ce que nous savons sur le métabolisme des acides gras. Le deuxième facteur donne une saturation élevée avec le poumon, le troisième est manifestement négligeable.

Il est permis de supposer que le taux cétonique des organes est soumis à un mécanisme régulateur commun qui ne coïncide point avec les fluctuations du même taux dans le plasma. Ce mécanisme trouve apparemment un réflet dans le premier facteur centroïde. Sa nature ne saurait être déterminée par une analyse mathématique: cette dernière pose un problème qui appelle une solution expérimentale.

E. Schreider

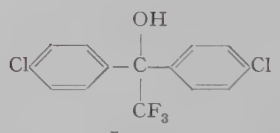
Laboratoire d'Anthropologie Physique de l'Ecole des Hautes Etudes, Paris, le 7 avril 1956.

Summary

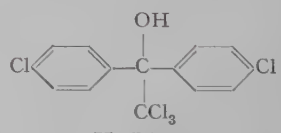
Ketone levels in the rat's organs (liver, muscle, lung, kidney, brain) do not seem to be correlated with the ketone level in the blood plasma. On the other hand, there are significant and high correlations between the ketone levels of different organs. These findings are not inconsistent with the generally accepted theory according to which blood distributes hepatic ketone bodies to the periphery. But ketone levels of the organs seem to be regulated not by variations of the plasma ketones, but by another common factor. At present, it is impossible to say how this regulation is effected.

The Action of Di-(p-chlorophenyl) trichloromethylcarbinol (DTMC) and Di-(p-chlorophenyl) trifluoromethylcarbinol on Houseflies

In course of a research program on DDT-synergists, an attempt was made to reactivate DDT against DDT-resistant houseflies either by keeping DDT residues in a semi-solid[1] or a liquid state[2], or with the help of compounds developed in these laboratories, as e.g. di-(*p*-chlorophenyl) trifluoromethylcarbinol[3] (I). It had been attempted[4] already in 1953 to prepare also the closely related di-(*p*-chlorophenyl) trichloromethylcarbinol (II, henceforward called DTMC). The synthesis of DTMC, m.p. 104–105° C, b.p. 225°/5 mm, has now been realized by chlorination of di-(*p*-chlorophenyl) methylcarbinol (DMC); full details will be published elsewhere.



I



II, DTMC

However, synergization of DDT probably does not represent a solution to the problem of DDT-resistant houseflies, due to (a) the speed of development of resistance to some DDT-synergist combinations[5], (b) reduced synergism in the order solution-, emulsion-, and wettable powderresidues, and (c) the inactivity or very short persistence of DDT-synergist residues against *highly* resistant

[1] S. Davidovici, Z. Levinson, and S. Reuter, WHO/Mal 37 (1950).

[2] K. R. S. Ascher, S. Reuter, and Z. H. Levinson, *Advances in Insecticide Research* (Jerusalem 1951), 18 p.; Chem. Abstr. *46*, 1698 (1952). – K. R. S. Ascher and S. Reuter, Riv. Parassitol. *14*, 115 (1953).

[3] E. D. Bergmann, A. S. Tahori, A. Kaluszyner, and S. Reuter, Nature *176*, 266 (1955). – A. Kaluszyner, S. Reuter, and E. D. Bergmann, J. Amer. chem. Soc. *77*, 4164 (1955).

[4] A. Kaluszyner and S. Reuter, J. Amer. chem. Soc. *75*, 5126 (1953).

[5] R. B. March, R. L. Metcalf, and L. L. Lewallen, J. econ. Ent. *45*, 851 (1952).

Table I. — Contact action of 1 g/sq.m. I, 1 g/sq.m. DDT + 1 g/sq.m. I, 1 g/sq.m. DTMC, 1 g/sq.m. DTMC + 1 g/sq.m. DDT, and 1 g/sq.m. DTMC + 1 g/sq.m. DDE on houseflies

4 × 10♀ flies, 2–3 days old; contact — 2 h; t = 27°C																
R-flies, % k.d. observed																
	I	DDT + I	DDT	DTMC				DTMC + DDT			DTMC + DDE			DDT		
Age of deposit in days	7	7	7	1	2	7	14	2	7	14	2	7	14	2	7	14
Contact in min																
10	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–
20	–	–	–	15	–	–	–	5	2	2	5	2	–	–	–	2
30	–	–	–	20	–	–	–	7	5	2	10	7	–	–	–	2
60	7	–	7	52	5	–	–	37	22	12	65	27	22	–	–	2
90	30	7	10	85	27	17	12	72	50	32	92	47	45	–	–	2
120	55	25	15	87	32	35	30	90	70	52	97	75	70	2	–	10
Σ% k.d.	92	32	32	264	64	52	42	211	149	100	269	158	137	2	0	18

T-flies, % k.d. observed					
	I	DDT + I	DDT	DTMC	DTMC + DDT
Age of deposit in days	7	7	7	7	7
Contact in min					
10	7	25	67	2	20
20	10	85	100	20	90
30	12	90	100	32	100
60	12	90	100	77	100
90	35	100	100	87	100
120	55	100	100	90	100
Σ % k.d.	131	490	567	308	510

Note: No k.d. in untreated controls

strains on *tarsal* contact (unless the synergist itself is a toxicant).

A highly DDT-resistant (R) and a nearly susceptible strain (T) of *Musca vicina* Macq. were exposed to residues from acetone in Petri dishes (continuous contact[6]). Since residues of DDT[7] and of some DDT-synergist combinations on glass tend to stay in the form of droplets (supercooling), R-flies were rushed over the deposits prior to the experiments until crystallization was complete. The results in the Tables are characteristic examples of numerous replications.

Although it has been reported[8] that I is an excellent DDT-synergist when tested by topical application, R-flies, on tarsal contact, were practically unaffected by a residue of 1 g/sq.m. DDT + 1 g/sq.m. I (Table I). The same results were obtained with 0·5 g/sq.m. DDT combined with either 0·05, 0·1, 0·25 or 0·5 g/sq.m. I. In the T-strain, no synergization of DDT by I was evident either, during continuous contact.— I had some slight fumigant properties, which could be demonstrated by a previously described method[9] with some slight modifications (flies above mosquito netting stretched between 2 Petri dish halves of equal size and facing each other, the deposit being on the lower one). R-flies, 2–3 days old, were thus exposed to the vapours only, at 27°C. The following % k.d. was recorded with deposits of 1 g/sq.m. I, 1 day, and 7 days (in brackets) old: 1–8 h: 0 (0); 9 h: 5 (2); 10 h: 5 (2); 11 h: 22 (2); 12 h: 50 (2); 13 h: 52 (20); 14 h: 70 (25); 15 h: 77 (35); 16 h: 85 (47); 17 h: 95 (67); 18 h: 95 (87); 19 h: 100 (92); 20 h: (95); 21 h: (100). The slight action exhibited by I itself on contact (Table I) was thus probably due partly to fumigant properties, which were apparently suppressed by addition of DDT to the residue.

The contact toxicity of DTMC (Table I) towards R-flies is only of very short duration, but its persistence is greatly prolonged by DDT. The enhanced activity of the DDT-DTMC residue (1:1, the same effect was demonstrated with 0·5 g/sq.m. DDT + 1·5 g/sq.m. DTMC and 0·25 g/sq.m. DDT + 0·75 g/sq.m. DTMC) is most probably not due to synergization of DDT by DTMC; it is much more likely that it is a "support"-effect exerted by DDT on DTMC. A similar "support"-effect was obtained, when other DDT-related compounds[10] (either active or inactive), as e.g. di-(*p*-chlorophenyl) dichloroethylene (DDE, see Table I), di-(*p*-tolyl) trichloroethane ("Methyl-DDT") and diphenyltrichloroethane, were combined with DTMC. Moreover, intermittent contact on 7 days old residues (glass jars) gave the following results, when R-flies were transferred after the contact into perforated cellophane bags[11] and observed for k.d. after 24 h:

1 h on 1 g/sq.m. DTMC + 1 g/sq.m. DDT:	52% k. d.
1 h on 1 g/sq.m. DTMC + 1 g/sq.m. DDE:	100% k. d.
1 h on 1 g/sq.m. DTMC, then 1 h on 1 g/sq.m. DDT:	no k. d.
ditto, but *vice versa*:	no k. d.
1 h on 1 g/sq.m. DTMC, then 1 h on 1 g/sq.m. DDE:	no k. d.
ditto, but *vice versa*:	no k. d.
1 h on 1 g/sq.m. DTMC:	no k. d.

DTMC is also much more active against T-flies than I. No synergization of DDT was apparent during continuous contact with this strain, either (Table I). DTMC did not exhibit any fumigant properties.— A closely related compound, di-(*p*-chlorophenyl) dichloromethylcarbinol[12], m.p. 108–109°C, was completely inactive against R-flies on continuous contact.

[6] C. Kocher, W. Roth, and J. Treboux, Anz. Schädlingsk. *26*, 65 (1953).

[7] K. R. S. Ascher and E. D. Bergmann, Bull. Res. Council Israel *3*, 255 (1953).

[8] A. S. Tahori, J. econ. Ent. *48*, 638 (1955).

[9] K. R. S. Ascher, Z. H. Levinson, P. H. Silverman, and A. S. Tahori, Riv. Parassitol. *15*, 45 (1954).

[10] No "support"-effect was obtained with di-(*p*-chlorophenyl) tribromoethane or diphenyl.

[11] F. Beran, Pflanzenschutzber. *11*, 151 (1953). – K. R. S. Ascher and C. Kocher, Exper. *10*, 465 (1954).

[12] J. Pepper and M. Kulka, J. Amer. chem. Soc. *72*, 1417 (1950).

R-flies brought into contact with DTMC or its DDT-"supported" combinations fall on their back and show recurrent tremors of the extended extremities ("Streck-tremor"). Flies kept in contact for long periods often have a blown-up abdomen due to swallowing of air. These symptoms of poisoning somewhat resemble those described for pyrolan[13].

Unfortunately, the action of DTMC and its combinations with DDT and related substances against resistant houseflies, compares very unfavorably with that of the newer phosphor insecticides as e.g. diazinon[6] or malathion (Table II), and it seems that the *insecticidal* properties of DTMC are of theoretical interest only. However, while this work was in progress, excellent *acaricidal* properties were claimed for α-trichloromethyl 4,4'-dichlorobenzhydrol, which seems to be the same compound as DTMC, but neither its mode of preparation, nor any physical properties have been described[14].

Table II. — Contact action of 0·25 g/sq.m. malathion on R-flies

4 × 10♀ R-flies, 2–3 days old; contact — 2 h; t = 27°C					
Age of deposit in days	1	7	14	21	28
Contact in min					
10	32	70	–	2	–
20	90	97	42	10	12
30	97	97	95	62	52
60	100	100	100	100	100
90	100	100	100	100	100
120	100	100	100	100	100
Σ % k.d	519	564	437	374	364

Note: No k.d. in untreated controls

We wish to thank Prof. G. G. Mer for helpful criticism.

The excellent technical help of Miss Dalia Roch is gratefully acknowledged.

S. Reuter[15] and K. R. S. Ascher[16]

Medical Research Laboratories, Medical Corps, Israel Defence Forces, Israel, April 5, 1956.

Résumé

L'action des carbinols di-(*p*-chlorophenyl) trifluorométhyl et di-(*p*-chlorophenyl) trichlorométhyl (DTMC) a été déterminée sur une souche très résistante de mouches par la méthode de contact. Le premier composé semble agir probablement grâce à de faibles propriétés fumigantes, alors que le second est toxique par contact. L'action toxique de ce dernier est sensiblement augmentée par l'addition du DDT, DDE ou autre composé organique de la série du DDT.

[13] R. Wiesmann and C. Kocher, Z. angew. Ent. *33*, 297 (1951).
[14] H. F. Wilson and J. S. Barker, 126th Meeting of American Chemical Society, Section 29A, 1954. – D. Asquith, J. econ. Ent. *48*, 329 (1955).
[15] Chemical development of DTMC.
[16] Biological evaluation.

The Effect of Formaldehyde on the Mutagenic Action of X-rays in *Drosophila**

Earlier investigations showed an increase of X-ray induced mutation rates after pretreatment with cyanide and azide in immature germ cells which are characterized by peak sensitivity to the mutagenic action of X-rays[1]. This result suggested that an increased production of hydrogen peroxide, due to inhibition of the cytochrome- and catalase enzyme systems, made the genetic material more susceptible to the effects of radiation. This assumption was further supported by the observation that pretreatment with an organic peroxide (dihydroxydimethyl peroxide) likewise significantly enhanced the mutagenic action of X-rays, notably in the same sensitive stage of spermatogenesis as pretreatment with cyanide and azide[2].

Because formaldehyde is also known to act as a catalase inhibitor[3] and presumably produces mutations via the formation of an organic peroxide[4], it was thought of interest to study the effects of formaldehyde pretreatment on the rate of X-ray induced mutations.

Material and methods.— The formaldehyde used was a BDH commercial preparation. All solutions were made with a 0·7% solution of sodium chloride in distilled water. The formaldehyde concentrations ranged from 0·033–0·050 Mols per litre. 2–3 day old Oregon-K males were injected intraabdominally with 0·28 mm³ of such solutions and were afterwards tested for the incidence of sex-linked lethals by means of the Muller-5 method, the first matings being started one day after treatment. To reduce the time interval between pretreatment and irradiation, the flies were injected in batches of about 30 individuals which were then placed immediately under the X-ray apparatus, the average time interval between injection and irradiation being 11 ± 8 min. The pretreated and X-ray control flies were differently marked with indian ink on the thorax which made possible the simultaneous exposure of both groups. X-radiation was administered by a General Electric, Maximar 100 Model of the Dermatology Clinic of the University of Utrecht. It was run at 100 KVP and 5 mA, at a dose rate of 244 r per min with 1 mm Al filtration (HVL = 1·3 mm Al).

To study the sensitivity pattern in the testes, treated males were remated to 3 fresh, virgin Muller-5 females at specific time intervals. The successive broods arising in this way then represent successively younger stages of spermatogenesis at the time of treatment[5]. No controls were used, because the spontaneous mutation rate in the Oregon-K stock rarely exceeds 0·2–0·3%.

Results.— The results of experiments with 2 different concentrations of formaldehyde are presented in Table I and Figure 1. In experiment 1 pretreatment with a concentration of 0·05 Molar which is hardly capable of raising the spontaneous mutation rate, caused a significant increase of the X-ray induced mutation rates in both the first and second broods. Pretreatment with an even weaker 0·042 M formaldehyde solution in experiment 2 likewise enhanced the mutation rates produced by the irradiation with a factor of from 1·60 to 1·85 in all 3 broods.

* This paper is dedicated to Prof. Dr. Jacob Seiler, Zürich, on the occasion of his 70th birthday.
[1] F. H. Sobels, Z. ind. Abst. Vererbungsl. *86*, 399 (1955).
[2] F. H. Sobels, Nature *177*, 979 (1956).
[3] K. G. Stern, Hoppe-Seyl. Z. *209*, 176 (1932).
[4] F. H. Sobels and J. W. I. M. Simons, Z. ind. Abst. Vererbungsl. (in press); Nature *177*, 979 (1956).
[5] Ch. Auerbach, Z. ind. Abst. Vererbungsl. *86*, 113 (1954).

Table I. Frequency of sex-linked lethals in 2–3 successive three-day broods from males which had been injected with solutions of formaldehyde prior to X-radiation, as compared with that from males which were irradiated or injected only.

Experiment	Formaldehyde concentration in Mols/l	Radiation dose in r	Sex-linked lethals – Days after treatment											
			1–4				4–7				7–10			
			n chrom.	%leth.	χ^2	*P*	*n* chrom.	%leth.	χ^2	*P*	*n* chrom.	%leth.	χ^2	*P*
F 1	0·050	–	363	0·8	–	–	360	0·3	–	–	388	0·5	–	–
R 1	–	1700	388	5·6			373	10·4			–	–		
					3·8	0·05			6·2	< 0·02			–	–
F–R 1	0·050	1700	478	9·9			268	17·5			–	–		
R 2	–	2200	338	6·5			341	9·1			306	3·6		
					5·9	< 0·02			7·4	< 0·01			1·3	0·2–0·3
F–R 2	0·042	2200	269	13·0			287	16·7			115	6·1		

For all χ^2 calculations corrections have been made allowing for the mutagenicity of formaldehyde alone in the corresponding broods.

In consideration of the low mutation rates induced by 0·05 M formaldehyde, no formaldehyde controls were taken for 0·042 M in experiment 2. The χ^2 for the difference between FR and R has been corrected for the frequency of mutations, as produced by 0·05 M formaldehyde by itself in experiment 1. Even then there remains a real excess of mutations in the first 2 broods of the pretreated series (FR_2).

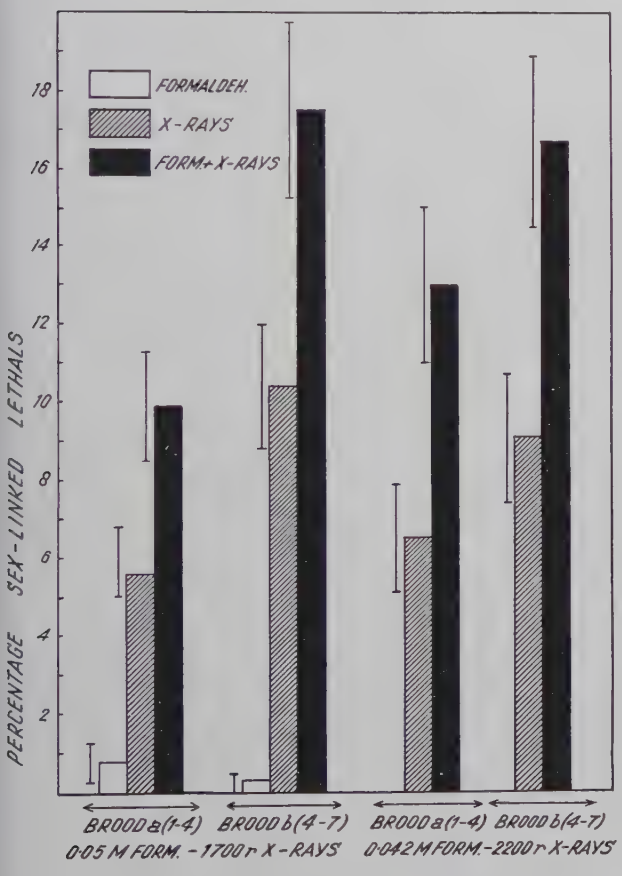


Fig. 1.—The frequency of sex-linked lethals in 2 successive broods (experiments 1 and 2) from males which had been injected with solutions of formaldehyde prior to X-radiation, as compared with that from males which were irradiated or injected only.

Formaldehyde thus resembles cyanide, azide and dihydroxydimethyl peroxide in potentiating the mutagenic action of X-rays. But its effects are different in that formaldehyde pretreatment raises the X-ray induced mutation rates in both the first and second broods, whereas in the other pretreatment experiments, the enhancement seemed specifically confined to stages sampled during the second brood only.

This finding raises the question whether formaldehyde which is known to have a mutagenic effect on mature sperm, similarly exerts a potentiating effect on X-ray induced mutation rates in this stage. Two further experiments were therefore done with daily brood changes during a period of 5 days after treatment. It was hoped that this method would result in a clearer separation of stages with different sensitivity. To ensure a maximum comparability, the two experiments were carried out within a short space of time, with males from the same sample and with the same 0·033 M formaldehyde solution. This procedure also had the advantage that one group of controls, receiving formaldehyde only, would be sufficient.

The results of the 2 separate experiments are presented in Table II. In view of their great similarity the data were pooled; this combined result is set out in Figure 2. First of all it can be seen that from the second day onwards formaldehyde is effective in causing a pronounced enhancement of the mutation rates induced by the radiation. No significant differences could be observed, however, for mutation rates of sperm sampled on the first day after treatment. It would seem therefore that fully mature sperm does not respond any more to the potentiating effect of formaldehyde on X-ray mutagenesis.

The possibility remains that the effect on the second day is different in nature from that on the later ones. Baker and von Halle[6] found that after irradiation in nitrogen, there is no difference between dominant lethal rates of sperm used up during the second and on the first day. Similarly the enhancement observed with formaldehyde on the second day might be due to prevention of recovery from the irradiation effects by the pretreatment.

The sharp increase of mutation rates in the subsequent broods suggests that this mating-scheme has been highly effective in selecting stages which become increasingly more sensitive to the mutagenic action of

[6] W. Baker and E. S. v. Halle, Proc. nat. Acad. Sci. Washington *39*, 152 (1953).

Table II. Frequency of sex-linked lethals in 5 successive one-day broods from males which had been injected with a 0·033 M solution of formaldehyde prior to X-radiation with 1700 r, as compared with that from males which were irradiated or injected only.

Experiment	Sex-linked lethals – Days after treatment																			
	1		2				3				4				5					
	n	%l	*n*	%l	χ^2	*P*	*n*	%l	χ^2	*P*	*n*	%l	χ^2	*P*	*n*	%l	χ^2	*P*		
Formaldehyde controls .	365	0·3	419	1·0	–	–	469	0·2	–	–	506	1·0	–	–	430	0·5	–	–		
R 3	504	5·4	409	4·6			318	4·4			332	12·3			121	17·4				
					–				6·31	<0·02			6·70	<0·01			–			
F–R 3	514	7·0	425	6·1			388	9·3			192	21·4			39	33·3				
R 4	446	4·5	436	3·0			350	3·7			325	13·9			113	27·2				
					10·16	<0·01			9·25	<0·01			–				–			
F–R 4	527	4·9	460	8·5			339	8·0			187	18·2			59	33·9				
$\sum$ R 3 + R 4	950	5·0	845	3·8			668	4·0			657	13·1			234	22·2				
					7·19	<0·01			15·48	<0·001			7·05	<0·01			4·76	<0·05		
$\sum$ F R 3 + F R 4 . . .	1041	6·0	885	7·4			727	9·3			379	19·8			98	33·6				

For all χ^2 calculations corrections have been made allowing for the mutagenicity of formaldehyde alone in the corresponding broods.

irradiation. A comparison between the mutagenic effects in the first and in the fifth broods gives a clear picture of the tremendous differences of sensitivity correlated with different stages of spermatogenesis.

It is also seen that in the X-ray controls the mutation rates in the second and third broods are slightly lower than in the first. This observation fits in with similar findings reported by a number of different workers[7]. Nordback recently showed that the observed decrease on the second day after the irradiation is not so much due to a further difference in sensitivity as to the effect of storing irradiated spermatozoa in the testes.

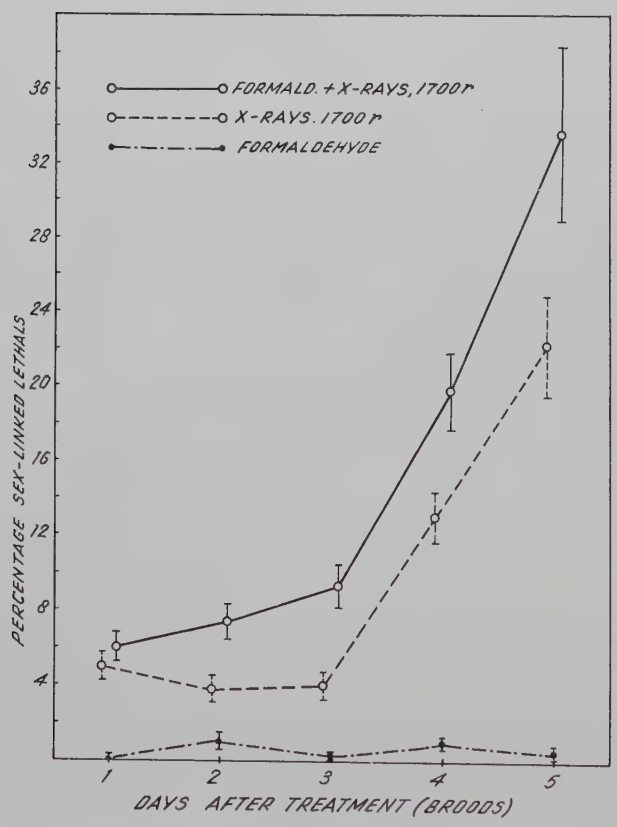


Fig. 2.—The frequency of sex-linked lethals in 5 successive one-day broods from males which had been injected with a solution of 0·033 M formaldehyde prior to X-radiation with 1700 r, as compared with that from males which were irradiated or injected only.

Discussion.— The data clearly show that pretreatment with low "submutagenic" concentrations of formaldehyde enhances the mutagenic action of X-rays. The possible interpretation of this finding may be considered next.

As mentioned before, it is known that formaldehyde inhibits catalase. In addition to this effect, two lines of evidence suggest that formaldehyde exerts at least part of its mutagenic action via the formation of peroxides. First, it could be shown that a given dose of formaldehyde produces a significantly greater number of mutations in males which had been pretreated with cyanide than in males which were not so pretreated. Second, it was found that dihydroxydimethyl peroxide, the organic peroxide formed by combining formaldehyde and hydrogen peroxide, acts as a fairly potent mutagen. Moreover, the pattern of sensitivity to the mutagenic action of this peroxide bears a marked resemblance to that found after injection of formaldehyde[8] (and unpublished data).

The hypothesis that an increased production of peroxides sensitizes the chromosomes to the effects of radiation thus seems further supported by the present findings on formaldehyde pretreatment. Like previous results, obtained after pretreatment with cyanide, azide and dihydroxymethyl peroxide, they suggest that the formation of peroxides may be involved in the mutagenic action of X-rays on the genetic material of *Drosophila*.

In contrast to the other chemicals, the effect of formaldehyde pretreatment is not so narrowly restricted to stages with peak sensitivity, but extends also to later stages of spermatogenesis. This finding might be con-

[7] W. Baker and E. S. v. Halle, Proc. nat. Acad. Sci. Washington *39*, 152 (1953). – J. D. Telfer and S. Abrahamson, Drosophila Information Service *28*, 161 (1954). – J. Mossige, Proc. 4th Int. Conf. Radiobiol., Cambridge, 1955 (in press). – K. G. Lüning, Proc. 4th Int. Conf. Radiobiol., Cambridge, 1955 (in press). – K. Nordback, Drosophilia Information Service *29*, 150 (1955).

[8] F. H. Sobels, Nature *177*, 979 (1956).

nected with the sensitivity of these stages to the mutagenic action of formaldehyde itself. After injection of formaldehyde in highly mutagenic concentrations, using daily brood changes, peak sensitivity was observed in sperm sampled on the second and third day after treatment (unpublished work). Another possibility is that formaldehyde speeds up sperm utilization by killing, or otherwise inactivating spermatozoa.

The author wants to express his thankfulness to Dr. Charlotte Auerbach for reading the manuscript and to Dr. Sheila Counce for correcting the English. He feels much indebted to Prof. Dr. J. J. Zoon who made the X-ray apparatus of his department available for this study. The technical assistance of Miss V. van den Hul is gratefully acknowledged. The investigation was supported in part by the Radiobiological Group of the Health Research Council T.N.O.

F. H. Sobels

Institute of Genetics of the State University, Utrecht, Netherlands, May 17, 1956.

Zusammenfassung

Vor einer Röntgenbestrahlung, deren Dosis von 1700 bis 2200 r variierte, wurde *Drosophila*-Männchen Formalinlösung in schwacher, „submutagener" Konzentration injiziert. Es zeigte sich, dass im Vergleich zu den Kontrollen, welche nur bestrahlt wurden, die Mutationsrate nach Formalin-Vorbehandlung signifikant gesteigert war. Dabei wurden spätere Entwicklungsstadien der Spermatogenese erfasst als nach einer Vorbehandlung mit Zyanid, Azid und einem organischen Peroxid; bei reifen Spermatozoen liess sich jedoch keine Zunahme der Mutationsrate feststellen. Die Befunde weisen darauf hin, dass ein erhöhter Gehalt an organischen Peroxiden in den vorbehandelten Zellen die Chromosomen empfindlicher macht für die mutagene Wirkung der Röntgenbestrahlung.

La culture de cellules tumorales sur des explants d'organes *in vitro*

Des cellules tumorales peuvent-elles envahir des organes ou fragments d'organes embryonnaires explantés *in vitro*, comme elles colonisent les tissus de l'organisme entier? J'ai abordé ce problème au moyen de la technique de culture organotypique de Wolff et Haffen[1]. Des fragments du sarcome de Souris S 180 ont été associés à des organes ou fragments d'organes embryonnaires de Poulet de 6 à 9 jours d'incubation. Les deux fragments se soudent intimement, comme le font des organes embryonnaires de Poulet et de Souris associés (Wolff[2]).

Au contact d'explants de différents organes, les cellules tumorales prolifèrent activement. Elles peuvent entourer l'organe d'une sorte de cortex dont les cellules extérieures s'exfolient. Elles pénètrent en même temps à l'intérieur par des voies d'invasion qui empruntent généralement le stroma conjonctif. Elles se divisent activement dans les tissus de l'hôte et forment soit des nodules compacts, soit des traînées orientées qui propagent la tumeur à grande distance de son point d'entrée.

Les organes qui se sont révélés les plus aptes à la prolifération tumorale (Fig. 1) sont le mesonephros, les gonades, le metanephros, le derme, le poumon, le périoste, les mésentères et les tuniques de l'intestin, la capsule et les cloisons du foie. Le mesonephros est particulièrement favo-

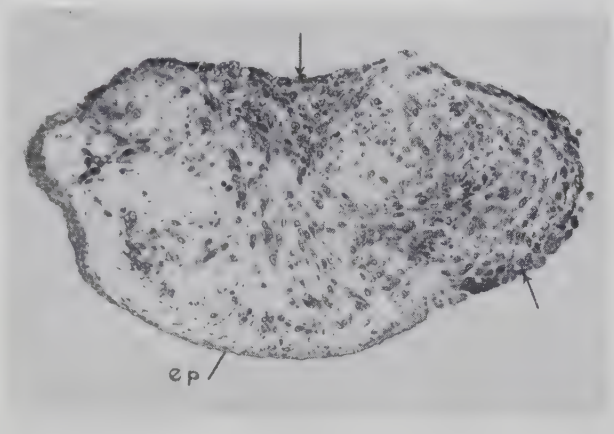


Fig. 1. Invasion de la paroi tégumentaire ventrale d'un embryon de Poulet, cultivée *in vitro*, par le sarcome S180 de Souris. Les cellules du sarcome peuvent être facilement distinguées grâce à leur taille et à leur coloration sombre. Elles envahissent pratiquement tout l'explant dont il ne subsiste localement que l'épiderme (ep.) et des cellules conjonctives éparses. (Les régions les plus riches en cellules tumorales sont indiquées par des flèches.) Grossissement × 122.

rable à la prolifération des cellules tumorales qui s'infiltrent dans les espaces intertubulaires (Fig. 2) et entourent les canalicules urinaires qu'ils finissent par envahir, après dégénérescence de leurs cellules. Les cellules tumorales montrent un aspect de fibroblastes fuselés, lorsqu'elles sont en migration; un aspect ovoïde ou arrondi quand

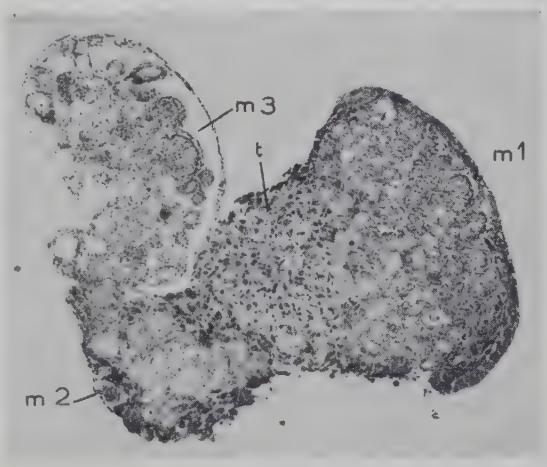


Fig. 2. Association d'un fragment de tumeur S180 de Souris avec plusieurs explants de mesonephros d'embryon de Poulet de 7 jours. L'explant tumoral se développe sur place au contact de deux des explants de mesonephros. L'invasion se fait par la périphérie des organes aussi bien que par leur face interne. Toutes les plages ou taches sombres sont des régions colonisées par des cellules tumorales. m1, m2: explants de mesonephros envahis par la tumeur. m3: explant encore intact. t: nodule tumoral développé aux dépens du fragment initial. Grossissement × 75.

elles sont groupées en amas. Les mitoses sont nombreuses, souvent aberrantes. L'organe peut être complètement envahi en l'espace de 2 à 4 jours. On peut ainsi propager

[1] Et. Wolff et K. Haffen, J. exp. Zool. *119*, 381 (1952); Texas Rep. Biol. Med. *10*, 463 (1952).

[2] Et. Wolff, Bull. Soc. zool. Fr. *79*, 357 (1954). – Et. Wolff et J. P. Weniger, J. Embr. exp. Morph. *2*, 161 (1954).

la souche tumorale par repiquages successifs en culture d'organes.

Dans le cas des autres organes, les cellules tumorales se multiplient au contact de l'explant de Poulet, formant un amas compact; elles s'infiltrent dans les tissus suivant la direction des strates conjonctives ou des tuniques musculaires. Dans les organes qui présentent un cloisonnement conjonctif, comme les gonades, l'invasion se fait le long des travées conjonctives. A la faveur de ces voies de pénétration, les cellules tumorales envahissent bientôt tout l'organe, en particulier le cortex ovarien, où elles ont tendance à prendre la place des ovogonies.

Ces résultats montrent qu'on peut cultiver et propager des cellules tumorales sur des organes explantés *in vitro*. Cette méthode donne la possibilité d'étudier les conditions de l'invasion cancéreuse d'un organe, indépendamment de la circulation et de toute connexion. Elle permet d'aborder, dans des conditions de simplicité qui ne sont jamais réalisées dans un organisme, les problèmes des affinités, de la stimulation et de l'inhibition des cellules tumorales. Elle peut orienter les recherches sur la sensibilité différentielle à certaines substances des cellules cancéreuses et des tissus qu'elles parasitent.

E. Wolff

Laboratoire d'Embryologie expérimentale, Collège de France, Paris, le 25 avril 1956.

Zusammenfassung

Setzt man in Organkulturen *in vitro* kleine Teilstücke eines S180-Maustumors mit embryonalen Organen des Huhnes zusammen, so wandern die tumoralen Zellen in die explantierten Organe über, bevölkern sie und vermehren sich aktiv.

Gewisse Organe wie Vorniere, Mesenterien, Darmwand, Kapsel der Leber, Haut, erweisen sich als besonders geeignet für die Einwanderung und die Vermehrung der tumoralen Zellen.

Tumor Necrosis After the Intra-Tumor Injection of Chemical Agents and Colloidal Radioisotopes

In laboratory and clinical investigations of the use of radioisotopes for the management of tumors, colloidal radioisotopes are injected directly into the tumor[1] with the objective of producing a maximum of tumor necrosis. In view of the increasing number of anti-tumor chemicals which have been successful in producing temporary clinical remission of neoplastic diseases[2], we have undertaken an exploratory study of the ability of a series of those agents to produce tumor necrosis after intratumor injection[3].

Sarcoma 180 tumors were injected on the 8th day after transplantation[4] to the axillary region of Swiss mice. The injectate was a freshly prepared solution or suspension of the agent in saline; each milliliter contained 50 mg of carbon black[7] to mark the intratumor site of deposition. For each agent, 0·03 ml was injected aseptically into each of 5 tumors. After the agent had remained in the tumor for the number of days shown in the Table, the animals were sacrificed and the tumors excised. The tumors were fixed in dilute formalin, sliced to find the region of maximum deposition of carbon black, and hematoxylin and eosin slides of sections from this region were prepared.

The extent of gross tumor necrosis was then determined by microscopic examination, with the absence of stained cell nuclei as the criterion for gross necrosis. This criterion was selected because of the objectivity and relative ease of determining the per cent of the tissue area in which the nuclei did not stain. Inasmuch as there was some variation in size of the injected tumors (within the range from 1·0 to 1·5 cm), the per cent necrosis was calculated on the basis of the necrosis in the 1·0 cm circle centering on the site of maximum car-

Sarcoma 180 Necrosis after Intra-Tumor Injection of Agents

Agent	mg per ml	Days	% Necrosis
methyl nitrogen mustard	0·07	1	48 ± 3
methyl nitrogen mustard	0·67	1	65 ± 4
methyl nitrogen mustard	0·57	3	81 ± 9
β-naphthyl nitrogen mustard[5]	50	3	90 ± 4
phenyl nitrogen mustard[6]	50	3	62 ± 4
p-chlorophenyl nitrogen mustard	50	3	69 ± 5
triethylene melamine	0·40	1	68 ± 2
triethylene melamine	6·7	1	80 ± 4
triethylene melamine	0·67	3	79 ± 7
triethylene phosphoramide	10	1	51 ± 2
triethylene phosphoramide	10	3	85 ± 6
yttrium 90 fluoride	25[a]	3	51 ± 7
chromic radiophosphate	5[a]	3	5 ± 3
cortisone acetate	50	2	14 ± 3
hydrocortone	20	2	42 ± 6
testosterone propionate	25	2	31 ± 4
methyl testosterone	10	3	42 ± 7
diethylstilbestrol	10	3	55 ± 6
estinyl estradiol	15	3	54 ± 5
aminopterin	3·3	2	20 ± 5
furadroxyl	67	2	38 ± 6
isoniazid	50	3	53 ± 6
p-aminosalicylate	67	2	4 ± 2
6-mercaptopurine	67	1	68 ± 2
6-mercaptopurine	83	3	84 ± 6
zinc peroxide	333	2	48 ± 5
p-menthane hydroperoxide	[b]	1	64 ± 2
p-menthane hydroperoxide	[b]	3	87 ± 4
colchicine	2·5	2	28 ± 4
urethane	67	1	69 ± 4
urethane	250	3	83 ± 5
myleran	0·8	1	20 ± 4
controls	saline	1	3 ± 2
controls	saline	3	3 ± 2

[a] Radioisotope concentrations are in millicuries per ml of injectate.
[b] 0·03 ml of liquid *p*-menthane hydroperoxide.

[1] R. H. Flocks, H. D. Kerr, H. B. Elkins, and D. A. Culp, J. Urol. *71*, 628 (1954).

[2] C. C. Stock, in: *Advances in Cancer Research*, Vol. 2 (Greenstein and Haddow, Academic Press, Inc., New York 1954), p. 426.

[3] Presented at the National Meeting of the American Association for the Advancement of Science, Berkeley, California, December 1954.

[4] The original transplant material was donated by Dr. John B. Field, Cancer Chemotherapy Laboratory, University of Southern California.

[5] Donated by: Dr. W. C. J. Ross, The Chester Beatty Research Institute, The Royal Cancer Hospital, London, S.W.3.

[6] M. E. Morton, Nucleonics *10*, 92 (1952). – S. W. Mayer and M. E. Morton, *Proceedings of the O.R.I.N.S. Conference on Rare Earths in Medicine* (1955) (in press).

[7] Sterling MT grade, Godfrey L. Cabot, Inc., Boston 16, Massachusetts.

bon deposition. Accordingly, the per cent necrosis data on the Table refer to gross necrosis that would be produced in a 1 cm tumor. The standard deviations are also tabulated.

As controls, a total of 24 tumors were injected with 0·03 ml of isotonic saline containing the usual proportion of carbon black. 12 tumors were excised after 1 day, 12 after 3 days. The average tumor necrosis produced by the saline injections was 3% with a standard deviation of 2%, for both the 1 day and 3 day time intervals.

Similar measurements of the necrotic effect of intratumorally injected methyl nitrogen mustard were carried out on Jensen sarcoma, in Sprague-Dawley rats. Three-hundreths of a milliliter of injectate, containing 0·67 mg per ml, were injected into each of 12 tumors. The average necrosis was 70% after 1 day, and 90% after 3 days (for the remaining 6 tumors).

Although the 3 aromatic nitrogen mustards listed in the Table were insoluble in saline, their effectiveness was comparable to the soluble agents. Also, β-naphthyl nitrogen mustard[8] and triethylene melamine, compounds which are normally administered orally to patients, were not less effective after intra-tumor injection than the two compounds which are generally administered intravenously, methyl nitrogen mustard and triethylene phosphoramide. Diethylstilbestrol and 6-mercaptopurine, which also have low solubility in saline were, nevertheless, relatively effective in producing tumor necrosis. The latter 2 compounds and β-naphthyl nitrogen mustard were individually tested for their ability to control the growth of Jensen Sarcoma in rats. Six tenths milliliter of a 50 mg/ml suspension of each compound were injected throughout the tumor on the 6th day after transplanting, and at three-day intervals thereafter. Each compound succeeded in keeping the tumor size down to 15–30% of the controls, and produced apparent disappearance of the tumor in some instances within 2 weeks – with survival periods longer than 6 months.

The intra-tumor administration of insoluble anti-tumor compound could reduce the systemic toxic effects frequently observed after the systemic administration of anti-tumor agents, since insolubility would ordinarily prevent rapid movement from the tumor. Of course, such administration of insoluble agents would be limited to the tumors for which radioisotopes have been used – bronchogenic, breast, prostate, cervical and other types of accessible tumors (and possibly for control of ascitic fluid formation). In principle, it would appear desirable to investigate the effectiveness of intra-tumor combinations of radioisotopes with one or more anti-tumor chemicals of the nitrogen mustard, hormone or anti-metabolite types.

S. W. Mayer and M. E. Morton

Radioisotope Research Unit, Veterans Administration Hospital, Long Beach, California, and the School of Medicine, University of California at Los Angeles, April 23, 1956.

Zusammenfassung

Mehrere klinisch wirksame, geschwulsthemmende Stoffe verursachten relativ rasch Nekrosen der Mäusesarkoma-180-Tumoren, wenn sie, entsprechend der klinischen Anwendung von radioaktiven Kolloiden, direkt in den Tumor injiziert wurden. Sowohl unlösliche, wie lösliche Verbindungen ergaben Nekrose. In den Tumor injiziertes 6-Merkaptopurin, Diäthylstilböstrol und β-Naphtyl-Stickstoff-Lost verminderten alle bei Ratten das Wachstum des Jensen-Sarkoms.

[8] A. A. Videbaek and S. Kaae, Acta med. Scand. *149*, 361 (1954).

Potentiation Action of Ibogaine (Bogadin TM) on Morphine Analgesia

Neostigmine[1], B-diethylaminoethyldiphenylpropylacetate hydrochloride[2], certain adrenocortical hormones such as DCA[3], and insulin[4] have been shown to potentiate morphine analgesia. Chlorpromazine prolongs the analgetic morphine effect[5] but does not necessarily enhance it[6]. Therefore, the reduction of morphine analgesia in mice pretreated with reserpine was a rather unexpected finding[5]. The combination effects of these phrenotropic agents with morphine prompted us to investigate the pharmacodynamic interactions of morphine with ibogaine, an indole alkaloid from *Tabernanthe iboga*[7] with characteristic central stimulant properties[8].

Potentiation of Analgetic Effect of Morphine Sulfate by Ibogaine HCl

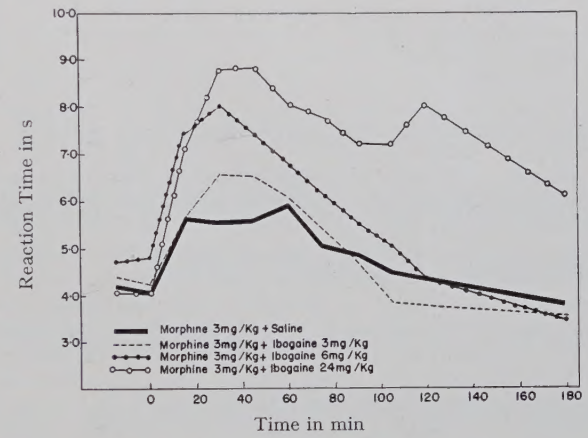


Fig. 1. – Comparison of average reaction time to a thermal pain stimulus applied to the mouse tail following subcutaneous injection of morphine alone and in combination with ibogaine. Each curve represents an average from a group of 10 mice. Prolongation of the reaction time following the combination of morphine and ibogaine becomes more pronounced as the dose of ibogaine increases.

Methods. – 370 female white mice (Webster strain) were tested for their reaction to pain by directing a beam of heat on the tip of their tails (Gross[9]). The reaction time was measured for each mouse as determined by the characteristic tail flick. Double readings ob-

[1] D. Slaughter and D. W. Nunsell, J. Pharmacol. exper. Therap. *68*, 104 (1940).

[2] L. Cook, G. Navis, and E. J. Fellows, J. Pharmacol. exper. Therap. *112*, 473 (1954).

[3] C. A. Winter and L. Flataker, J. Pharmacol. exper. Therap. *103*, 93 (1951).

[4] F. Gross and H. Kaufmann, Helv. physiol. Acta *12*, 284 (1954).

[5] J. A. Schneider, Proc. Soc. exper. Biol. Med. *87*, 614 (1954).

[6] J. Kopera and A. K. Armitage, Brit. J. Pharmacol. Chemother. *9*, 392 (1954).

[7] J. Delourme-Houde, Thesis, University of Paris (1944).

[8] J. A. Schneider and E. B. Sigg, Ann. N. Y. Acad. Sci. (in press).

[9] F. Gross, Helv. physiol. Acta *5*, C31 (1947).

tained for each group of mice were averaged and the standard deviations of the control values calculated. Analgetic effects following drug injection were either expressed in prolongation of the reaction time or by calculating the number of mice of each group showing significant analgesia. Significant analgesia was assumed when a reading of an individual animal was greater than the mean reaction time of the corresponding group by a factor of 2·32 times standard deviation[10].

Quantitative comparison of the analgetic effect of morphine alone and in combination with ibogaine. Each experiment represents a group of 10 mice.

Drug	% of Mice Showing Significant Analgesia		
	Control %	at Peak of Effect %	at 180 min %
Morphine 3 mg/kg	0	50	0
Morphine 3 mg/kg + Ibogaine 6 mg/kg.	0	80	0
Morphine 3 mg/kg + Ibogaine 24 mg/kg	0	100	50

Results.— Ibogaine hydrochloride alone did not produce any significant analgetic effect in doses up to 40 mg/kg subcutaneously. However, when given in combination with morphine, a clearcut potentiation of the analgetic effect of morphine was demonstrated (Fig. 1). A standard dose of 3 mg/kg of morphine was combined with different doses of ibogaine, the ratio of the two drugs being 1:1, 1:2, and 1:8. As the amount of ibogaine was increased the analgetic effect became more pronounced. Statistical analysis of these data revealed that the percentage of animals showing significant analgesia at the peak of the drug effect increased from 50 to 100% as increasing amounts of ibogaine were added to a standard dose of 3 mg/kg of morphine (Table). Furthermore, the analgetic effect lasted longer following combinations of higher doses of ibogaine with morphine. Similar potentiations of analgesia could be obtained following combinations of ibogaine with ketobemidone, codeine and Demerol. However, ibogaine did not enhance the analgetic effect of aminopyrine. Parallel to the pronounced potentiating effect of ibogaine on morphine analgesia, an increase in toxicity could be observed when both drugs were administered simultaneously. Figure 2 represents percent of death in mice following single doses of morphine and ibogaine as well as after a combination of equal doses of each drug. As morphine and ibogaine had similar toxicities, a combination of both drugs increased their respective toxicities about five-fold.

Discussion.— Recent experiments performed by Stephen[11] confirmed the potentiating effect of ibogaine on morphine analgesia in human beings. Since ibogaine by itself does not produce analgesia, a simple additive effect can be excluded. One may therefore assume that it interferes with the metabolic process involved in the phenomenon of morphine analgesia. There is one point of attack that both morphine and ibogaine may have in common. According to Bernheim and Bernheim[12], Eadie[13], and Wright and Sabine[14], morphine markedly inhibits cholinesterase activity. Slaughter and Nunsell[1] showed in addition that the analgetic action of morphine can be enhanced by neostigmine. Ibogaine was reported to inhibit cholinesterase activity by Vincent and Sero[15], and was shown to produce a brainstem

The Effect of Various Doses of Morphine, Ibogaine and a Combination of Both on Lethality in Mice.

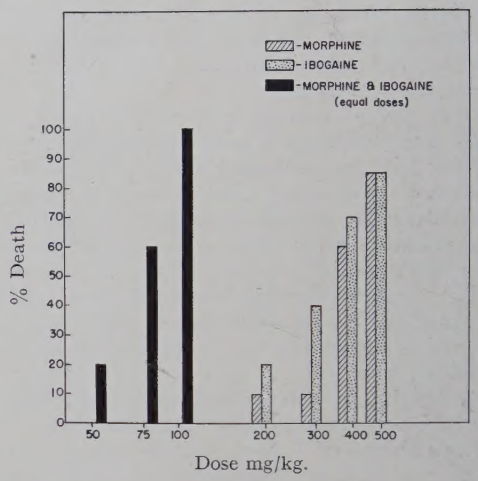


Fig. 2.— Demonstration of lethal effects of morphine, ibogaine and a combination thereof in mice. Doses graphed on logarithmic scale. Note the marked increase in toxicity of both drugs when administered in equal doses simultaneously.

arousal phenomenon, a cholinergic process which can be inhibited by atropine[8]. Provided that the cholinesterase inhibition by morphine is linked to its analgetic effect, the action of both drugs on the same enzyme may well offer an explanation for the potentiation action of ibogaine described in this paper.

J. A. Schneider and Marie McArthur

Research Department, Ciba Pharmaceutical Products Inc., Summit, New Jersey, May 28, 1956.

Zusammenfassung

Ibogain-hydrochlorid zeigte an der Maus einen ausgesprochen potenzierenden Effekt auf die analgetische Wirkung von Morphin und morphinähnlichen Analgetika und erhöhte, bei derselben Tierart, auch die Toxizität von Morphin. Die analgetische Wirkung von Aminopyrin hingegen wurde selbst durch hohe Dosen von Ibogain nicht beeinflusst.

[10] J. Ipsen, Acta pharm. tox. *5*, 321 (1949).
[11] R. Stephen, Personal Communication.
[12] F. Bernheim and M. L. C. Bernheim, J. Pharmacol. exper. Therap. *57*, 427 (1936).
[13] G. S. Eadie, J. biol. Chem. *138*, 597 (1941).
[14] C. I. Wright and J. C. Sabine, J. Pharmacol. exper. Therap. *78*, 373 (1943).
[15] E. Vincent and M. Sero, C. r. Soc. biol. *136*, 612 (1942).

Die pharmazeutischen
Spezialitäten «Sandoz»
sind das Ergebnis
intensiver chemischer,
pharmakologischer
und medizinischer
Forschung.
Sie geniessen
das Vertrauen
des Arztes
in der ganzen Welt.

Sandoz A.G. Basel

Chemische Werke

AUS DEM BIRKHÄUSER VERLAG BASEL / STUTTGART

XIV^e Congrès International de Chimie Pure et Appliquée – XIVth International Congress of Pure and Applied Chemistry – XIV. Internationaler Kongress für Reine und Angewandte Chemie.

Zürich, 20.–28. VII. 1955. 5 Hauptvorträge und 9 Sektionshauptvorträge in drei Sprachen. (*Experientia Supplementum II*) Ganzleinen, 283 Seiten. Fr./DM 32.– (1955).

Internationale Kommission zum Schutze des Rheins gegen Verunreinigung.

Bericht der Expertenkommission über die physikalisch-chemische Untersuchung des Rheinwassers.

1. Serie Juni 1953 bis Juni 1954, in deutscher und französischer Sprache. Broschiert, 53 Seiten, 22 Figuren und 17 Tabellen. Fr./DM 8.30 (1956).

Die Grundlagen der Anstrichwissenschaft.

Von Dr. A. V. Blom, 386 Seiten mit 171 Figuren. Ganzleinen Fr./DM 44.70. Broschiert Fr./DM 40.55 (1954).

Röntgenographisch-analytische Chemie.

Möglichkeiten und Ergebnisse von Untersuchungen mit Röntgeninterferenzen in der Chemie. Von Professor Dr. E. Brandenberger. 287 Seiten, 121 Figuren. Ganzleinen Fr./DM 31.20 (1946).

Das Polarisationsmikroskop.

Eine Einführung in die mikroskopische Untersuchungsmethodik durchsichtiger kristalliner Stoffe für Mineralogen, Petrographen, Chemiker und Naturwissenschafter im allgemeinen. Von Professor Dr. C. Burri. 310 Seiten mit 168 Figuren und 4 Tafeln. Ganzleinen Fr./DM 34.10. Broschiert Fr./DM 29.95 (1950).

Rationelle Metallanalyse.

Ausgewählte chemische Analysenmethoden für Aluminiumlegierungen, Bleilegierungen, Kupferlegierungen, Magnesiumlegierungen, Zinklegierungen, Zinnlegierungen. Von Dr. Ing.-Chem. A. Cohen. 416 Seiten, 29 Figuren und 54 Tabellen. Ganzleinen Fr./DM 47.85 (1948).

Prozenttabellen organischer Verbindungen – Tables of Percentage Composition of Organic Compounds – Tables des compositions centésimales des combinaisons organiques.

Von Dr. H. Gysel. 662 Seiten. Kunstleder Fr./DM 125.– (1951).

Die theoretischen Grundlagen der analytischen Chemie.

Von Professor Dr. G. Hägg. 200 Seiten mit 26 Figuren. Ganzleinen Fr./DM 22.90. Broschiert Fr./DM 18.70 (1950).

Chemische Analyse der Gesteine und silikatischen Mineralien.

Von Professor Dr. J. Jakob. 180 Seiten mit 10 Figuren. Ganzleinen Fr./DM 18.70. Broschiert Fr./DM 15.60 (1952).

Carotinoide.

Von Professor Dr. P. Karrer und Dr. E. Jucker. 388 Seiten mit 31 Figuren und 2 Farbtafeln. Ganzleinen Fr./DM 47.85. Broschiert Fr./DM 43.70 (1948).

DDT – Das Insektizid Dichlordiphenyltrichloräthan und seine Bedeutung.

In drei Bänden. Herausgegeben von Dr. Paul Müller. Band I: 300 Seiten mit 53 Figuren. Ganzleinen Fr./DM 37.50 (1955). Band II erscheint Ende 1956.

Grundlagen der Stereochemie.

Von Professor Dr. P. Niggli. 283 Seiten mit 207 Figuren. Ganzleinen Fr./DM 37.45. Broschiert Fr./DM 33.30 (1945).

Fouriersynthese von Kristallen und ihre Anwendung in der Chemie.

Von Professor Dr. W. Nowacki. 237 Seiten mit 120 Figuren. Ganzleinen Fr./DM 34.30. Broschiert Fr./DM 30.15 (1952).

Ins Innere von Kunststoffen, Kunstharzen und Kautschuken.

Von E. V. Schmid. Zweite erweiterte Auflage. 206 Seiten mit 130 Figuren und 21 Tabellen. Ganzleinen Fr./DM 19.25 (1949).

Vollständiges Verlagsverzeichnis der wissenschaftlichen Publikationen sowie Spezialprospekte der einzelnen Werke können durch den Verlag bezogen werden.